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Key Advances in Medicine

January 2017

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ATHEROSCLEROSIS IN 2016

Advances in new therapeutic targets for atherosclerosis

W. H. Wilson Tang and Stanley L. Hazen

In 2016, advances in atherosclerosis research were focused on the discovery and validation of new targets with genetic and mechanistic links to atherothrombotic heart disease. Novel targets include proteins involved in glycoprotein recognition and clearance, regulators of triglyceride-rich particle metabolism, inflammatory pathways that impair efferocytosis, and the gut microbiome.

Extending beyond genomic explorations that have successfully identified proatherosclerotic pathways such as *LPA* and *PCSK9*, several landmark papers this year reported on the identification of novel and rare genetic variants that highlight the involvement of additional processes in lipid metabolism which influence atherosclerotic risk (FIG. 1). Nioi and colleagues described genetic determinants that contribute to variations in the level of atherogenic lipoproteins[1]. Apolipoprotein B-containing particles can be captured globally by measuring non-HDL cholesterol (non-HDLc; calculated by deducting HDL cholesterol from total cholesterol). Non-HDLc is superior to LDL cholesterol in predicting coronary artery disease (CAD) risk, and the year 2016 marked the important discovery of a genetic association between variants affecting non-HDLc and CAD risk, and the protein asialoglycoprotein receptor 1 (ASGR1)[1]. ASGR1 is a lectin subunit of the transmembrane protein asialoglycoprotein receptor that has a critical role in serum glycoprotein homeostasis. In genetic discovery studies that initially involved approximately 400,000 Icelandic individuals, which were then validated in case–control studies from five European ancestry cohorts of approximately 300,000 individuals, carriers (approximately 1 in 120 Icelandic individuals) of a noncoding 12-basepair deletion in intron 4 of *ASGR1* were found to have reduced non-HDLc levels, and an accompanying marked reduction in risk of CAD[1]. Carriers of this loss-of-function genetic variant had a mean 15 mg/dl reduction in non-HDLc, a ~34% reduction in myocardial infarction risk, and a lifespan prolonged by 1.5 years compared with noncarriers[1]. The mechanisms underlying the pronounced atheroprotective effects of this variant, however, are unclear. This study thus reported that loss-of-function mutations in *ASGR1* result in non-HDLc reductions and marked protection from CAD, and identify *ASGR1* as a potential therapeutic target for the treatment and/or prevention of CAD[1].

Hypertriglyceridaemia is another well-established cardiovascular risk factor. The important role of angiopoietin-related protein 4 (ANGPTL4) in modulating triglyceride levels by inhibiting lipoprotein lipase (LPL), and in reducing the risk of CAD were validated in two separate large-scale genomic analyses in 2016 (REFS 2,3). Carriers of loss-of-function variants of *SVEP1* (p.D2702G) and *ANGPTL4* (p.E40K) had lower triglyceride levels than noncarriers; these mutations were also associated with protection from CAD[2]. Furthermore, a loss-of-function variant mutation in *LPL* (p.D36N) was associated with increased CAD risk, whereas a gain-of-function variant (p.S447*) was associated with reduced risk of CAD[2]. In an independent investigation reported this year, carriers of the E40K variant and 13 other monoallelic inactivating mutation variants of *ANGPTL4* all had lower triglyceride levels and higher HDLc levels than noncarriers, and were less likely to have CAD[3]. In addition, monoclonal antibody inhibition of the ANGPTL4 protein in both mouse and nonhuman primate models resulted in a reduction in triglyceride levels[3].

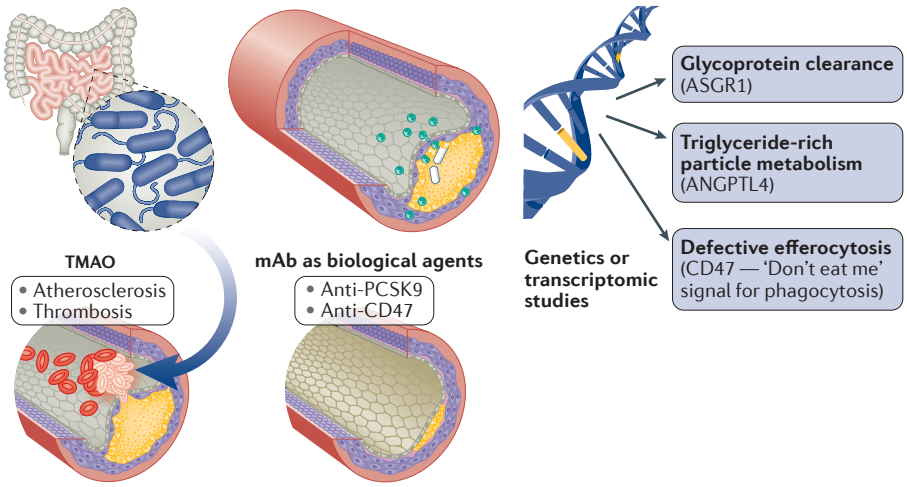

Figure 1 | **Newly discovered mechanistic pathways leading to development and progression of atherosclerosis.** Large-scale genomic studies revealed rare variants that alter the function of novel proteins involved in glycoprotein clearance (asialoglycoprotein receptor 1 [ASGR1]), triglyceride-rich particle metabolism (angiopoietin-related protein 4 [ANGPTL4]), and defective efferocytosis (CD47), which are linked to coronary artery disease. Gut microorganism-associated metabolism of dietary nutrients that are abundant in Western diets lead to the generation of the metabolite trimethylamine *N*-oxide (TMAO) , which is associated with enhanced coronary atherosclerotic plaque burden, heightened platelet responsiveness, and thrombotic complications. Therapeutic interventions with antibodies against PCSK9 in humans reduce LDL-cholesterol levels, inhibit coronary atherosclerosis progression, and promote plaque regression, whereas anti-CD47 therapy in animal models provides proof-of-concept validation for inhibition of atherosclerosis. mAB, monoclonal antibodies.

Key advances

- Asialoglycoprotein receptor 1 (ASGR1) can regulate non-HDL-cholesterol levels and influence the risk of coronary artery disease (CAD)[1]
- Variants in the *ANGPTL4* gene are linked to hypertriglyceridaemia and CAD risk[2,3]
- PCSK9 inhibition in the setting of intensive statin therapy can decrease LDL-cholesterol levels, reduce coronary atherosclerotic plaque progression, and induce significant reversal of atherosclerosis[5]
- An increase in the gut microorganism-dependent metabolite trimethylamine *N*-oxide is associated with coronary atherosclerotic burden, and linked to heightened platelet responsiveness and thrombosis potential[8,9]

Despite the strong inverse association between HDLc and CAD risk, results from therapeutic intervention trials targeting HDLc levels have yielded frustratingly negative findings. Perhaps some of the confusion surrounding HDLc was clarified through genomic analyses published this year of rare variants in individuals with high HDLc. Mice with a *Scarb1* knockdown have markedly elevated HDLc levels and paradoxically increased risk of atherosclerosis[4]. Targeted sequencing of coding regions of lipid-modifying genes in 328 individuals with high plasma HDLc levels led to the identification of a homozygote mutation for a loss-of-function variant (P376L) in *SCARB1* that impairs post-translational processing of the scavenger receptor class B member 1 protein and abrogates selective HDLc uptake in transfected cells[4]. Carriers of this *SCARB1* variant showed paradoxically elevated HDLc and an increased risk of CAD.

Progress has also been made this year in interventional studies for the treatment of atherosclerosis. Findings from the GLAGOV study[5] suggest that PCSK9 inhibition might reverse coronary atherosclerosis via incremental LDL-cholesterol lowering, even in the presence of aggressive statin therapy. Statin-treated patients ($n = 938$) with angiographic CAD were prospectively randomly assigned to subcutaneous evolocumab (a PCSK9 inhibitor) or placebo for 76 weeks and assessed by serial intravascular coronary ultrasonography[5]. Compared with placebo, the evolocumab group had lower LDL-cholesterol levels (as low as 20 mg/dl), had a greater decrease in atheroma volume, and showed plaque regression in a greater proportion of patients, without serious adverse effects. Although the study was not powered to assess clinical outcomes, major adverse cardiac events were numerically reduced with evolocumab therapy. These findings suggest that PCSK9 inhibition in the setting of intensive statin therapy can influence plaque progression and foster reversal of atherosclerosis.

Further mechanistic links between inflammation and atherosclerosis have also been explored[6]. Advanced lesions at risk of rupture are characterized by accumulation of diseased vascular cells and apoptotic cellular debris; why these cells are not cleared within atheroma remains unknown. Upregulation of CD47, an antiphagocytic molecule (a 'don't eat me' signal) known to render malignant cells resistant to programmed cell removal (efferocytosis), occurs during atherogenesis (FIG. 1). Impaired efferocytosis might have a pathogenic role in cardiovascular disease. In a 2016 study, CD47 was upregulated in both human coronary and carotid atherosclerotic vessels, presumably via a tumour necrosis factor (TNF)-α and NFκB1-dependent process that results in inhibition of efferocytosis[6]. Moreover, administration of CD47-blocking antibodies reversed the defect in efferocytosis, normalized the clearance of diseased vascular tissue, and ameliorated atherosclerosis in multiple mouse models through the TNFα-driven programmed cell removal process[6].

Multiple advances were reported this year on the role of the gut microbiome in atherothrombosis. A link between gut microorganisms and atherosclerotic plaque development was first established in 2011, in which untargeted metabolomic and functional studies led to the discovery of the atherogenic metabolite trimethylamine *N*-oxide (TMAO)[7]. In a 2016 study of patients with stable CAD undergoing coronary angiography and plaque characterization, elevated plasma level of TMAO was shown to predict coronary atherosclerotic burden more powerfully than traditional cardiac risk factors[8]. Microbial production of TMAO was also reported to serve as a novel mechanism linking diet and specific gut microbiota with induction of heightened platelet responsiveness and enhanced thrombosis potential *in vivo* in both humans and other animals[9]. Initial clinical studies ($n = 4{,}007$) reported that plasma levels of TMAO independently predicted the risk of thrombotic events, and subsequent mechanistic studies showed that TMAO enhanced submaximal stimulus-dependent platelet activation by multiple agonists through augmented Ca^{2+} release from intracellular stores[9]. Complementary animal-model studies employing dietary choline or TMAO, germ-free mice, and microbial transplantation collectively confirmed a role for gut microbiota and TMAO in modulating platelet hyper-responsiveness and thrombosis potential *in vivo*, and identified microbial taxa associated with plasma TMAO and thrombosis potential[9]. In another study, nonlethal inhibition of the rate-limiting microbial enzyme involved in generating TMAO from dietary choline was also shown to serve as a novel approach for the treatment of atherosclerosis[10]. These studies highlight the potential of targeting TMAO levels for the prevention and treatment of atherothrombosis.

In summary, numerous notable advances in atherosclerosis research were made in 2016. The power of genomics and other 'omics' technologies for discovery of new pathways linked to CAD development has begun to be realized, and has the potential to expand the number of therapeutic targets for atherosclerosis.

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S.L.H. is named as inventor on pending patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. He is also a paid consultant for Esperion and P&G, and has received research funds from Astra Zeneca, P&G, Pfizer Inc., Roche Diagnostics, and Takeda. S.L.H. has also received royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from Cleveland HeartLab, Esperion, Frantz Biomarkers, LLC, and Siemens. W.H.W.T. declares no competing interests.

GENETICS OF CHD IN 2016

Common and rare genetic variants and risk of CHD

Daniel I. Swerdlow and Steve E. Humphries

Much of the progress in cardiovascular genetics in 2016 has been driven by next-generation sequencing studies, and the clinical utility of knowing an individual's genotype for predicting their risk of cardiovascular disease is gaining credibility, both for monogenic and polygenic disorders. Additionally, phenotype data are increasingly abundant, although databases linking genotype with clinically relevant phenotypes require optimization.

Next-generation sequencing (NGS) has driven major advances in our understanding of monogenic causes of elevated LDL-cholesterol (LDL-C) levels and premature coronary heart disease (CHD) in familial hypercholesterolaemia (FH), focusing on the three proven FH-causing genes, *APOB*, *LDLR*, and *PCSK9*. NGS has a detection rate approaching 100% and a false-positive rate of almost 0%, but NGS is currently too expensive for population-scale screening. Although FH prevalence has historically been estimated at 1/500 in outbred populations, the true prevalence of FH-causing mutations now seems to be between 1/250 and 1/300. In Denmark, 98,098 participants from the Copenhagen General Population Study[1] were genotyped for three common *LDLR* mutations and the most common *APOB* mutation[1]. The prevalence of the four mutations was 1/565, accounting for about 39% of all pathogenic mutations in the country, and representing an estimated prevalence of FH-mutation carriers of 1/217 in the general population[1].

In the UK, Wald *et al.* aimed to identify adults with FH by measuring cholesterol levels in 10,094 children at routine immunization visits (median age 12.7 months)[2]. The investigators identified 45 children with FH; 37 had total cholesterol levels >5.31 mmol/l (205 mg/dl; 95th percentile) and a detected FH mutation, and eight had repeated LDL-C recordings ≥5.90 mmol/l (228 mg/dl; 99th percentile) and no detected mutation. Mutation carrier prevalence was 1/273 (37/10,094). We previously demonstrated that such individuals without a monogenic cause of FH probably have a polygenic aetiology for their FH phenotype, with co-inheritance of a greater than average number of common LDL-C-raising alleles of modest effect[3]. When testing parents of children with confirmed FH, Wald *et al.* identified 40 parents who also met the criteria for FH diagnoses[2], demonstrating the feasibility of the approach in primary care. In the UK, in patients with FH in whom a monogenic cause is found, approximately 93% have a mutation in *LDLR*, 5% in *APOB*, and 2% in *PCSK9*. Mutations in other genes have been proposed, but none has been independently confirmed. By contrast, 40% of the detected FH mutations in the Wald study were the *APOB* (p.R3527Q) mutation — a recognized cause of less severe FH than most *LDLR* mutations. Universal screening, therefore, identified many individuals with a genetic predisposition to milder hypercholesterolaemia, but who might be at less elevated risk of CHD than *LDLR* mutation carriers. Thus, although universal screening has value in identifying new families with FH, this approach can also identify individuals at comparatively low risk of cardiovascular disease, and guide decision-making in statin therapy where the risk–benefit balance is equivocal.

The elevated risk of CHD in patients with FH with a detected mutation was confirmed in a population study[4]. Khera *et al.* used NGS for the known FH genes in 20,485 individuals without CHD, of whom 1,386 (6.7%) had LDL-C levels >4.9 mmol/l (190 mg/dl), and of these, 24 (1.7%) had a known FH mutation. Individuals with an LDL-C level >4.9 mmol/l and no FH mutation had a six-fold higher risk of CHD than individuals with an LDL-C level <3.7 mmol/l (130 mg/dl) and no mutation, but individuals with both an LDL-C level >4.9 mmol/l and an FH mutation had a 22-fold higher risk. This increased risk is explained by the substantially higher accumulated 'LDL-C burden' in patients who have had genetically-determined, lifelong, high LDL-C levels.

Dilated cardiomyopathy is found in up to 1/250 in the general population, and is commonly caused by truncating mutations in the titin gene (*TTN*). *TTN*-truncating variants occur in about 1% of the general population, but not all carriers develop dilated cardiomyopathy. To explore the mechanisms of

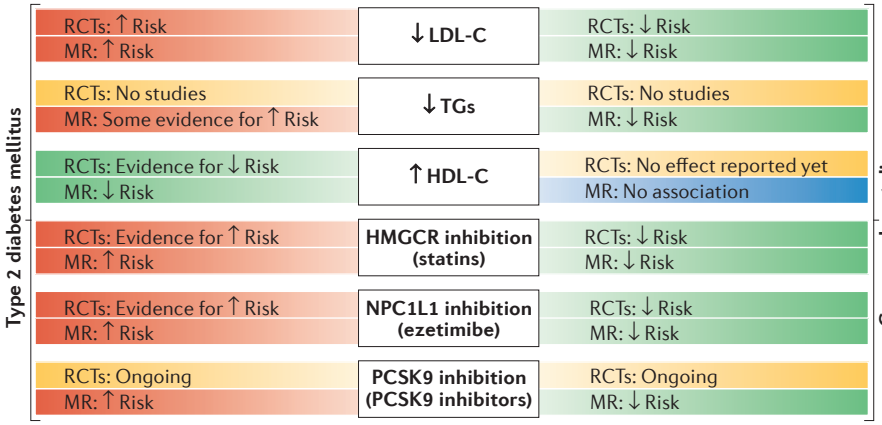

Figure 1 | **Lipid levels, lipid-modifying drugs, and risk of type 2 diabetes mellitus and CHD.** Evidence from randomized clinical trials (RCTs) and Mendelian randomization (MR) studies on the relationship between commonly measured lipid fractions — LDL cholesterol (LDL-C), triglycerides (TGs), and HDL cholesterol (HDL-C) — or lipid-modifying drug targets — 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), the essential enzyme in the cholesterol-synthesis cascade and inhibited by statins[7]; Niemann–Pick C1-like protein 1 (NPC1L1), inhibited by ezetimibe[9]; and proprotein convertase subtilisin/kexin type 9 (PCSK9), the main protein involved in LDL-receptor degradation during receptor recycling[10] — and risk of type 2 diabetes mellitus and coronary heart disease (CHD).

Key advances

- Prevalence of familial hypercholesterolaemia has now been established convincingly by next-generation sequencing and by genotyping in European, population-based studies to be roughly 1/250, twice as common as previously thought[1]
- Common truncating mutations in the gene encoding titin increase the risk of dilated cardiomyopathy, but only under stress conditions[5]
- A genetic risk score combining common single-nucleotide polymorphisms identified in genome-wide association studies showed clinical utility in identifying individuals at high risk of coronary heart disease (CHD), which can be offset with healthy lifestyle choices[6]
- Mendelian randomization studies have shown the directionally consistent effect of genetically-lowered or pharmacologically-lowered LDL-cholesterol levels in increasing risk of type 2 diabetes mellitus, while reducing risk of CHD, demonstrating a causal role of low LDL-cholesterol levels[8,10]

this 'variable penetrance', Shafer *et al.* used rat models and human cardiac imaging and genomic data[5]. *TTN*-truncating variants caused nonsense-mediated degradation of the mutant protein and altered cardiac metabolism in the rat model, and cardiac physiology was normal in young mutant rats but became impaired during stress. To define cardiac effects in the general population, Shafer *et al.* sequenced *TTN* in 1,409 healthy volunteers, and examined cardiac morphology and function with cardiac magnetic resonance. *TTN*-truncating variant carriage was associated with a phenotypic continuum of eccentric cardiac remodelling from healthy to severe disease[5]. As with FH, rare, highly penetrant mutations lead to severe disease, but less deleterious variants in the same gene cause a spectrum of cardiac dysfunction.

Using a genetic risk score (GRS) with 50 variants identified in genome-wide association studies (GWAS) to be associated with risk of CHD, Khera *et al.* compared genetically-determined and lifestyle-related risk of CHD in three prospective, USA and Swedish cohorts ($n \approx 56{,}000$; 5,103 CHD events)[6]. Healthy lifestyle was a composite of no current smoking, no obesity, regular physical activity, and healthy diet. Results were consistent across studies and between ancestral groups, and risk of incident CHD was 91% higher among individuals in the highest compared with the lowest GRS quintile[6]. The association between GRS and a family history of premature CHD was modest but significant. Unsurprisingly, an unhealthy lifestyle (absence of at least three of the four factors above) was related to a substantially higher (≈80%) risk of CHD, and the genetic and lifestyle risk scores had essentially cumulative effects. Individuals at high genetic risk who had a healthy lifestyle had lower overall risk of CHD than those with high genetic risk and an unhealthy lifestyle. In a substudy of 4,260 individuals, both genetic and lifestyle factors were associated with coronary artery calcification[6].

The debate over the relationship between blood lipids and risk of CHD and type 2 diabetes mellitus (T2DM) continues to intensify as results of outcome trials of novel proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors are awaited. Little doubt remains that reducing LDL-C levels diminishes the risk of CHD, and doing so with statins increases the risk of T2DM[7] (FIG. 1). White *et al.* used Mendelian randomization to explore the causal relationships between the three major lipid fractions — HDL cholesterol (HDL-C), LDL-C, and triglycerides — and risk of CHD and T2DM[8]. Importantly, these analyses were executed with novel methods and with publicly available GWAS data. As expected, both LDL-C and triglycerides had positive causal relationships with risk of CHD, but higher HDL-C was not associated with lower risk of CHD after accounting for pleiotropy of the genetic instruments[8]. Higher LDL-C and HDL-C levels were associated with lower risk of T2DM, suggesting that altered lipid fractions *per se*, rather than specific therapeutic mechanisms, can modulate glucose homeostasis.

The risk of T2DM associated with individual, therapeutic, lipid-modulatory mechanisms remains an area of interest[9]. Given the now-confirmed increased risk of T2DM with statins, attention rests on whether a similar risk increase will be observed in phase III trials of PCSK9 inhibitors. Identification of functional gene variants in proposed cardiovascular disease aetiological pathways has allowed focused exploration through Mendelian randomization of causal contributions of cardiovascular disease-associated lipid traits. Schmidt *et al.* showed that common *PCSK9* variants, used as proxies for pharmacological inhibition of the enzyme, were associated with lower LDL-C levels, higher blood glucose levels, higher body weight, and higher risk of T2DM (OR 1.29, 95% CI 1.11–1.50 per 1 mmol/l reduction in LDL-C level)[10]. These findings were confirmed in other genetic analyses, and emphasize the importance of diabetes outcomes in ongoing PCSK9 inhibitor phase III trials.

The year 2016 has brought substantial advances in our understanding of the genetic architecture of cardiovascular disease, although much uncharted territory remains, perhaps most strikingly in common, nonmonogenic heart failure. The HERMES consortium on heart failure genetics is set to address this issue in 2017 and its findings, along with those of many other ongoing efforts, are hotly awaited.

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D.I.S. has been a consultant to Pfizer. S.E.H. declares no competing interests.

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ARRHYTHMIAS IN 2016

Arrhythmia treatment — evidence catching up with technology

Benjamin A. Steinberg and Jonathan P. Piccini

Clinical cardiac electrophysiology has evolved rapidly over the past 2 decades. Although the fast pace of technical and therapeutic advances has occasionally outpaced evidence supporting widespread effectiveness, the highlights of electrophysiology research in 2016 illustrate the emergence of robust evidence for implementation of several important therapies.

The implantable cardioverter–defibrillator (ICD) has been available for >2 decades, but our understanding of the patients who benefit most from primary prevention ICD therapy continues to evolve. Historically, randomized trials have provided the most robust evidence and support for its use in patients with stable ischaemic cardiomyopathy complicated by symptomatic heart failure. However, on the basis of the findings from the SCD-HeFT study[1], primary prevention ICDs are also routinely used in patients with nonischaemic cardiomyopathy[2]. Nonetheless, the clinical benefit of ICDs in patients with nonischaemic cardiomyopathy is less certain. The publication of the DANISH trial[3] in early 2016 provided additional evidence to help guide primary prevention therapy in this population. The investigators randomly assigned 1,116 patients with nonischaemic cardiomyopathy and left ventricular ejection fraction <35% to usual clinical care with or without an ICD. The overall study showed no significant benefit of ICD therapy for the primary end point of all-cause mortality at median follow-up of 67.6 months. However, there are important caveats to consider when evaluating these results, particular when comparing with earlier studies of primary prevention ICD therapy in nonischaemic cardiomyopathy[3]. First, guideline-directed medical therapy for heart failure was ideal: >90% of patients received β-blockers and angiotensin-converting-enzyme inhibitors, and nearly two-thirds received mineralocorticoid-receptor antagonists. Despite their proven benefits, <30% of patients with heart failure receive mineralocorticoid-receptor antagonists in clinical practice[4]. More importantly,

> “… clinicians often turn to catheter ablation in patients with medically refractory VT, despite limited data…”

nearly 60% of patients overall received cardiac resynchronization therapy — a therapy with proven survival advantage in this population[5]. Additionally, findings from the DANISH study[3] showed substantial heterogeneity across patient age groups — younger patients seem to benefit from ICD therapy, and the risk of sudden cardiac death was significantly reduced in the overall trial population. Taken together, these data indicate that overall treatment of patients with nonischaemic cardiomyopathy has improved dramatically, including both better pharmacological and device-based interventions. Primary prevention ICDs are likely to be beneficial in this context, in which competing risks of non-sudden cardiac death are low. Additional studies should help to identify how to tailor ICD therapy to best balance the risks and benefits.

Given that ICDs prevent sudden cardiac death when ventricular tachycardia (VT) or ventricular fibrillation has occurred, prevention of recurrent ventricular arrhythmias is a critically important goal. Antiarrhythmic therapy has been shown to be beneficial, but is frequently inadequate and clinicians often turn to catheter ablation in patients with medically refractory VT, despite limited data from head-to-head randomized comparisons between antiarrhythmic therapy and catheter ablation. Investigators in the VANISH trial[6] enrolled 259 patients with ischaemic cardiomyopathy complicated by VT despite antiarrhythmic drug therapy. They were randomly assigned to ablation ($n = 132$) or escalated drug therapy ($n = 127$; amiodarone or amiodarone plus mexiletine), and followed up for a mean of 27.9 months for the primary composite end point of death, VT storm, or appropriate ICD shock[6]. Importantly, the protocol allowed for broad approaches in the ablation arm (FIG. 1), including activation mapping and substrate-based ablation in patients with haemodynamically unstable VT, and recommended targeting of all induced VTs. Patients assigned to ablation had a significant reduction in the primary end point (HR 0.72, 95% CI 0.53–0.98, $P = 0.04$), which was primarily driven by arrhythmia reduction[6]. Therefore, the VANISH trial[6] findings provide important confirmatory evidence for the use and effectiveness of catheter ablation to treat refractory ventricular arrhythmias in patients with ischaemic cardiomyopathy. Future trials should clarify whether catheter ablation might be preferred to antiarrhythmic drugs for first-line therapy given the risks and toxicities associated with these medications.

The past year has also brought additional evidence to guide management of the most common sustained arrhythmia, atrial fibrillation (AF). Occasionally, AF manifests in the setting of a perceived ‘reversible’ cause, such as acute medical illness, thyrotoxicosis, or cardiac surgery. In this setting, AF treatment has generally been conservative, with few data to guide management. In early 2016, the Cardiothoracic Surgical Trials Network published the results of a randomized trial of rate control versus rhythm control for postoperative AF[7]. Notably, one-third of patients without AF before the procedure subsequently developed AF after elective bypass or valve surgery ($n = 695$). These patients were then

Key advances

- Primary prevention with implantable cardioverter–defibrillators for nonischaemic cardiomyopathy should be individualized on the basis of competing risks[3]
- Catheter ablation of ventricular tachycardia should be recommended for patients with ischaemic cardiomyopathy and ventricular tachycardia refractory to drug therapy[6]
- Conservative management is appropriate for patients with postoperative atrial fibrillation complicating cardiac surgery; however, long-term surveillance is important given increased risks of future atrial fibrillation[7]
- Cryoballoon ablation seems to be as safe and effective as radiofrequency ablation for the treatment of paroxysmal atrial fibrillation[10]

randomly assigned to either rate-control medications only or rhythm control with amiodarone and cardioversion as needed. However, roughly one-quarter of each group crossed over to the opposite treatment arm. In total, 24% of patients in the rhythm-control group did not receive the full course of amiodarone and reverted to rate-control medications, and 26.7% of patients in the rate-control group eventually received amiodarone and/or direct current cardioversion[7]. The primary end point of hospitalization days within 60 days of randomization was not significantly different between the two groups. Perhaps more importantly, >80% of all patients did not have AF after discharge, and >90% of all patients were in a stable rhythm without AF after 30–60 days. Though the trial was not without limitations, the overall data provide support for a conservative approach in these patients, with strong reassurance that AF is unlikely to recur during short-term follow-up. However, additional data are needed to clarify the use of long-term surveillance, given that patients who develop AF after cardiac surgery are at high risk of recurrent AF.

The use of catheter ablation to treat symptomatic recurrent AF is increasing, and guidelines have evolved to include catheter ablation as first-line treatment in selected patients[8]. Ablation technologies have also improved, allowing for more efficient and effective pulmonary vein isolation, and now include cryoballoon catheters. This approach previously compared favourably to antiarrhythmic therapy among patients with symptomatic paroxysmal AF[9], but until this year, there were few trials comparing cryoablation with standard radiofrequency ablation. In 2016, the FIRE AND ICE trial[10] reported the noninferiority of the cryoballoon approach compared with radiofrequency ablation. The investigators randomly assigned 762 patients with drug-refractory, symptomatic, paroxysmal AF to ablation with either the cryoballoon or traditional radiofrequency ablation method; the primary end point was the first arrhythmic recurrence after a standard 90-day blanking period (mean follow-up of 1.5 years). The noninferiority margin was predefined at a hazard ratio of 1.43, and the trial met this threshold for equivalent efficacy (34.6% for cryoballoon versus 35.9% for radiofrequency ablation; HR 0.96, 95% CI 0.76–1.22, $P<0.001$ for noninferiority)[10]. Importantly, the safety end point of death, stroke, or transient ischaemic attack, or treatment-related adverse events (including phrenic nerve palsy), was not significantly different between the groups. These data provide reassurance that a potentially simpler ablation approach might be equally safe and effective in selected patients. However, the trial included only patients with paroxysmal AF, and although the mean duration of AF was >4 years, the patients' left atrial diameters were fairly small (40 mm) and the overall cohort was fairly young (70% were aged ≤65 years), with low CHA_2DS_2-VASc scores (1.8–1.9)[10]. Cryoablation in older, higher-risk patients with symptomatic, longstanding, persistent AF is unlikely to be as effective. Additionally, both radiofrequency ablation and cryoablation technologies have evolved considerably during the course of this trial, and now include contact force-sensing radiofrequency catheters, and a redesigned, second-generation cryoballoon catheter.

In summary, 2016 brought us several well-conducted clinical trials representing some of the best-quality evidence in contemporary clinical cardiac electrophysiology. Future trials in the coming years should build on their progress, helping clinicians to apply these technologies where they are most likely to have the greatest effect.

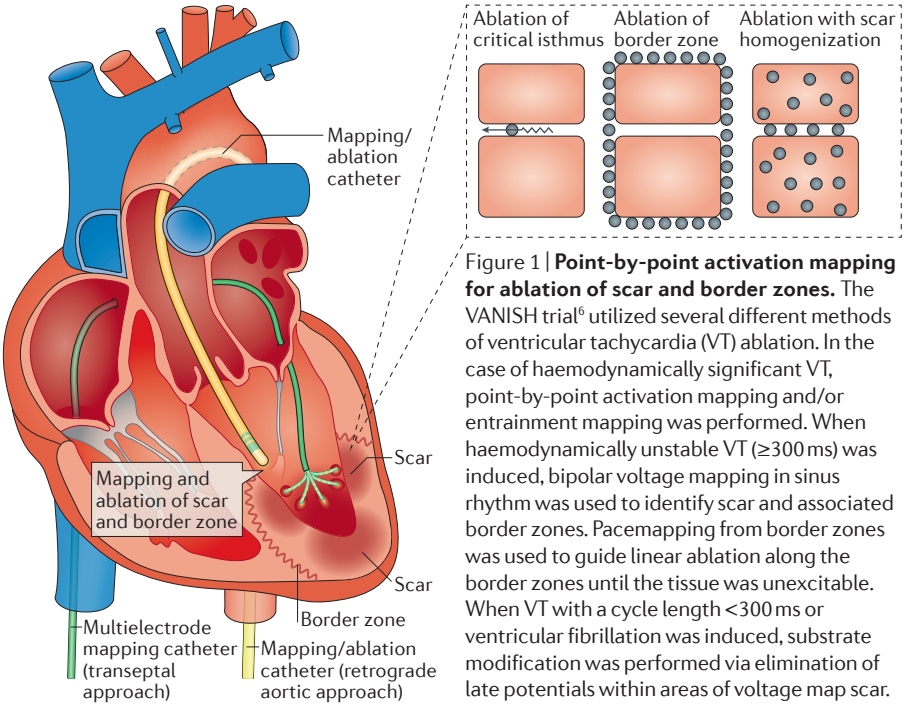

Figure 1 | **Point-by-point activation mapping for ablation of scar and border zones.** The VANISH trial[6] utilized several different methods of ventricular tachycardia (VT) ablation. In the case of haemodynamically significant VT, point-by-point activation mapping and/or entrainment mapping was performed. When haemodynamically unstable VT (≥300 ms) was induced, bipolar voltage mapping in sinus rhythm was used to identify scar and associated border zones. Pacemapping from border zones was used to guide linear ablation along the border zones until the tissue was unexcitable. When VT with a cycle length <300 ms or ventricular fibrillation was induced, substrate modification was performed via elimination of late potentials within areas of voltage map scar.

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ACUTE CORONARY SYNDROMES IN 2016

Assessing strategies to improve patient management

Ron Waksman

The leading studies in 2016 on acute coronary syndromes focused on strategies of acute coronary management, from the influence of revascularization timing on outcomes, to selection of second-generation antiplatelet therapy and the utility of monitoring platelet function in patients at high risk of coronary artery disease.

In 2016, the leading randomized clinical trials in acute coronary syndromes (ACS) were designed to address strategies to improve management. Among these strategies were the optimal time for revascularization and optimal time for stenting in patients with ST-segment elevation myocardial infarction (STEMI). These questions have been addressed in two important studies: the FRISC-II[1,2] and DANAMI 3-DEFER[3] trials. Second-generation antiplatelet agents (prasugrel and ticagrelor) showed superiority over clopidogrel for patients with ACS in the TRITON-TIMI 38 (REF. 4) and the PLATO[5] studies; however, the two agents had not been directly compared in a randomized study. Accordingly, the PRAGUE-18 trial[6] was conducted to compare prasugrel and ticagrelor antiplatelet therapy in patients with STEMI. Finally, the role of monitoring antiplatelet therapy to optimize the anticoagulation regimen for patients undergoing percutaneous coronary intervention (PCI) with stenting remains in question after studies demonstrated that the strategy did not add value to the management of these patients[7,8]. The ANTARCTIC trial[9] was designed to explore the utility of platelet-function monitoring for elderly patients with ACS undergoing coronary stenting.

Over the past 2 decades, no consensus has emerged on the optimal time for intervention in patients with ACS. The FRISC-II study[1] was among the first to address this important question. The FRISC-II trial[1], conducted in Scandinavia from 1996 to 1998, showed that early invasive treatment within 7 days of admission for the index event in patients with non-ST-segment elevation ACS significantly reduced the risk of death and myocardial infarction (MI) compared with noninvasive treatment[1]. After 15 years of follow-up, the investigators now report that

> “The FRISC-II study … supports the need for early intervention in patients with ACS”

early revascularization postponed death or new MI (the primary end point) by an average of 18 months (mean of 549 days; 95% CI 204–888 days; $P = 0.002$) compared with the noninvasive strategy[2]. This effect was larger in patients who were nonsmokers, had elevated troponin T levels, or high concentrations of growth differentiation factor 15. The difference in the primary end point was driven mainly by delay of a new MI, because the early difference in mortality was not sustained over time. Early revascularization postponed death or readmission to hospital for ischaemic heart disease by a mean of 1,128 days (95% CI 830–1,366 days; $P < 0.0001$), a benefit that was similar across all subgroups. The FRISC-II study[1] suggests that an early invasive strategy is the preferred option in most patients with non-ST-segment elevation ACS, and supports the need for early intervention in patients with ACS. As with STEMI, the focus should be on providing early intervention to optimize outcomes.

PCI of the culprit lesion in patients with STEMI and multivessel disease is the common practice. PCI can often be associated with thrombotic complications and embolization that might complicate the procedure. This issue led to the hypothesis that deferring stent implantation in these patients might improve outcomes. The DANAMI 3-DEFER trial[3] was designed to assess whether deferred stenting reduced the risk of impaired coronary blood flow and improved the clinical course of patients with STEMI compared with conventional PCI. Among 1,215 patients with chest pain lasting ≤12 h and ST-segment elevation in whom a stabilized TIMI flow 2–3 was achieved with minimal lesion manipulation, deferred stenting did not improve the composite primary end point of all-cause death, hospitalization for heart failure, recurrent infarction, or target-vessel revascularization after a median of 42 months compared with immediate stenting. Mortality and hospitalization for heart failure numerically favoured deferred stenting, but this difference was compensated by a significantly higher rate of target-vessel revascularization, mainly owing to 11 patients who had either recurrent symptoms or ST-segment elevation and returned prematurely for stent implantation. At 18 months after treatment, an echocardiographic evaluation in two-thirds of the patients showed left ventricular function was slightly better in the deferred stenting group than in the conventional PCI group (60% versus 57%; $P = 0.04$). Procedure-related complications were not significantly different between the groups. The investigators concluded that routine deferred stent implantation did not reduce the incidence of death, heart failure, MI, or repeat revascularization in patients with STEMI compared with conventional PCI[3]. These results are not surprising, and show that fear of complications should not deter us from stenting patients with STEMI. Instead of deferring stenting, other measures should be explored to prevent distal embolization, including minimizing manipulation at the culprit lesion by direct stenting and optimal anticoagulation. These strategies should be assessed in future studies.

In the past decade, two second-generation antiplatelet drugs — prasugrel and ticagrelor — became available for the treatment of patients with STEMI. Both drugs were found

Brain light/Alamy Stock Photo

Key advances

- The 15-year follow-up of the FRISC-II trial showed that early invasive treatment reduced the risk of death and myocardial infarction compared with noninvasive treatment in patients with non-ST-segment elevation acute coronary syndrome[2]
- Deferred stenting in patients with ST-segment elevation myocardial infarction (STEMI) does not reduce the occurrence of all-cause death, myocardial infarction, hospitalization for heart failure, or target-vessel revascularization[3]
- The findings from the PRAGUE-18 trial to compare prasugrel and ticagrelor antiplatelet therapy in patients with STEMI cannot be substantiated owing to the lack of statistical power and the early termination of the trial[6]
- The results from the ANTARCTIC trial do not support the recommendation in current guidelines to perform platelet-function testing in patients at high risk of coronary artery disease[9]

to be superior to clopidogrel for the treatment of this condition[4,5]. To compare the two medications directly, the PRAGUE-18 trial[6] investigators evaluated prasugrel head-to-head with ticagrelor in patients with acute STEMI who had undergone PCI. However, the trial was halted prematurely because an interim analysis showed no difference between the two drugs for the primary end point of death, reinfarction, urgent target-vessel revascularization, stroke, serious bleeding requiring transfusion, or prolonged hospitalization at 7 days. The occurrence of the secondary end point of cardiovascular death, nonfatal MI, or stroke at 30 days was also similar between both groups. The investigators concluded that these findings do not support the hypothesis that either prasugrel or ticagrelor is more effective or safer than the other in preventing ischaemic and bleeding events in the acute phase of MI in patients treated with primary PCI[6]. However, the results of this study cannot be substantiated owing to the lack of statistical power and the early termination of the trial, and the rationale of a cutoff at 7 days rather than 30 days for the primary end point is in question. The results of this study confirm previous indirect comparisons between the two drugs and indicate a drug class effect, suggesting that physicians can use either prasugrel or ticagrelor for the treatment of patients with STEMI.

Platelet-function testing has been proposed to guide antiplatelet therapy in patients undergoing PCI. Nevertheless, previous studies such as the GRAVITAS[7] and ARCTIC[8] trials showed no benefit of platelet-function testing with treatment modification in a low-risk patient population and in stable patients undergoing elective stenting. However, the low-risk patient population in the ARCTIC trial[8] might have led to low event rates that were insufficient to demonstrate a significant difference, and the antiplatelet therapy used was predominantly clopidogrel. In the ANTARCTIC trial[9], the investigators sought to show a benefit of platelet-function testing and treatment modification in a high-risk patient population by using prasugrel, a more potent antiplatelet agent. A total of 877 patients with ACS, aged ≥75 years, who had undergone coronary stenting, were assigned to receive prasugrel (5 mg per day), with 442 patients randomly assigned to no monitoring or adjustment of medication, and 435 patients randomly allocated to monitoring with a two-step, platelet-function testing with the VerifyNow $P2Y_{12}$ assay (Accriva Diagnostics, USA) at 14 days after randomization and at 14 days after treatment adjustment, with treatment adjustment after each monitoring. Patients with high on-treatment platelet reactivity (≥208 $P2Y_{12}$ reaction units) had their prasugrel dose increased to 10 mg per day; patients with low on-treatment platelet reactivity (≤85 $P2Y_{12}$ reaction units) were switched to clopidogrel (75 mg per day); and patients with 85–208 $P2Y_{12}$ reaction units continued to receive prasugrel 5 mg per day. After the first platelet-function testing, 182 patients (42%) were in the target range of platelet inhibition. After the two-step treatment adjustment strategy, an additional 105 patients reached the prespecified target of platelet inhibition. A total of 171 patients (39%) in the monitoring group were switched from prasugrel to clopidogrel for low on-treatment platelet reactivity, and only 16 patients (4%) had high platelet reactivity. Although 5 mg prasugrel was safe for the elderly population, the primary end point (a composite of cardiovascular death, MI, stroke, stent thrombosis, urgent revascularization, and further bleeding complication at 1 year), as well as rates of major and minor bleeding, occurred at a similar rate in both arms of the study. The trial investigators concluded that treatment adjustment on the basis of platelet-function monitoring did not improve the clinical outcome of elderly patients treated with coronary stenting for ACS[9]. These findings do not support the recommendation in current guidelines to perform platelet-function testing in patients at high risk of coronary artery disease, and will probably result in a change to the guidelines and a decline in the use of platelet-function testing to individualize antiplatelet therapy.

Overall, the 2016 data on ACS support early and complete intervention (if possible) in patients with ACS and STEMI. Either of the second-generation antiplatelet therapies, prasugrel or ticagrelor, should be utilized in these patients, and probably routine platelet-function monitoring during PCI no longer has a role in the management of these patients.

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Competing interests statement
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MULTIPLE MYELOMA IN 2016

Fresh perspectives on treatment and moments of clarity

Prashant Kapoor and S. Vincent Rajkumar

Data obtained in the past year underscored the benefit of a triplet regimen comprising bortezomib, lenalidomide, and dexamethasone for patients with newly-diagnosed multiple myeloma, and have provided high-level evidence supporting the safety of adding daratumumab to standard-of-care doublets for those with relapsed and/or refractory disease. As a result, achieving minimal residual disease-negativity at any stage of myeloma is now a realistic possibility.

In 2015, we witnessed the approval of four new antimyeloma agents — panobinostat, elotuzumab, ixazomib, and daratumumab — with vastly different mechanisms of action. By any measure, 2015 was a groundbreaking year for the multiple myeloma (MM) community; an encore performance would be no easy task, but 2016 undeniably passed muster as another year of breakthroughs, with the publication of a series of practice-changing studies[1–4]. One overarching theme emerging from three major trials with results published in the past year is that the use of three-drug combination (triplet) regimens provides prolonged durations of remission compared with those achieved with doublet regimens in patients with either newly diagnosed or relapsed MM. A second important implication is that very deep therapeutic responses can be achieved in both of these patient populations, necessitating a revision of the assessment of response to therapy in MM[5].

Until recently, considerable confusion surrounded the optimal approach to therapy for patients with newly diagnosed MM[6]. Results from a few randomized trials had shown survival benefits with certain novel regimens, but in these trials, newer regimens were compared with older melphalan-containing regimens[7]; data showing an unequivocal survival benefit from trials that compared two modern regimens were not available. In the past year, we finally received evidence for a clear 'winner': the results of the Southwest Oncology Group (SWOG) S0777 study[1], a phase III trial in 525 patients with newly diagnosed MM, decisively demonstrated marked improvements in the overall response rate (ORR), progression-free survival (PFS), and overall survival with the use of a bortezomib, lenalidomide, and dexamethasone (VRd) regimen compared with lenalidomide plus dexamethasone (Rd; TABLE 1). Importantly, the findings demonstrated a median overall survival in excess of 6 years with use of the VRd regimen (75 months, compared with 64 months with Rd)[1], highlighting a major improvement in the outcome of patients with MM. As a result, this triplet regimen is our recommended standard of care for initial therapy (www.mSMART.org).

Impressive as the results of the SWOG S0777 trial[1] are, they do not fully capture the progress that has been made in MM therapy since that trial completed accrual in early 2012. Notably, we now have several new active regimens with which to treat relapsed disease[8], and thus the median overall survival observed in the S0777 cohort might underestimate the true therapeutic benefits that patients with MM can expect today. In this regard, daratumumab, a fully human IgGκ monoclonal antibody targeting CD38, exemplifies the advances made to a much greater extent than any other new development. Daratumumab was initially approved by the FDA in 2015 based on the results of a small phase II study[9] that demonstrated the impressive activity of monotherapy with this agent in patients who had exhausted virtually all other available treatment options. The implications were obviously vast, with the results serving as a 'springboard' for the initiation of trials to evaluate a host of daratumumab-based combination regimens. That the consequences of such integration would yield unprecedented results became evident only when the findings of the CASTOR[2] and POLLUX[3] studies were released in rapid succession in 2016.

Table 1 | **Comparison of outcomes with triplet vs doublet myeloma treatment regimens**

Trial	Study interventions	ORR (MRD– rate)	Median DOR (months)	Median PFS (months)	Median OS (months)	Practice points
S0777 (REF. 1)	VRd* vs Rd*	**82% vs 72%** (NA)	**52 vs 38**	**43 vs 30 (HR 0.71)**	**75 vs 64 (HR 0.70)**	VRd‡ is the new standard of care for newly diagnosed myeloma
CASTOR[2]	DVd* vs Vd	**83% vs 63%** (NA)	**NR vs 8**	**NR vs 7.2 (HR 0.39)**	NR vs NR (HR 0.77)	• DVd‡ is an important new option for relapsed myeloma, especially lenalidomide-refractory disease • DVd is most suitable after first or second relapse
POLLUX[3]	DRd* vs Rd*	**93% vs 76% (22% vs 5%)**	NR vs 17	**NR vs 18.4 (HR 0.37)**	NR vs 20 (HR 0.64)	• DRd is an important new option for relapsed myeloma, especially in patients who are not refractory to lenalidomide • DRd is most suitable after first or second relapse

Statistically significant differences are in bold. DOR, duration of response; DRd, daratumumab, lenalidomide, and dexamethasone; DVd, daratumumab, bortezomib, and dexamethasone; HR, hazard ratio; MRD –, minimal residual disease-negative; NA, not available; NR, not reached; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; Rd, lenalidomide and dexamethasone; VRd, bortezomib, lenalidomide, and dexamethasone. *Continuously, until progression or intolerable effects. ‡Cumulative neurotoxicity can prevent long-term use of bortezomib, and can be ameliorated via once-weekly subcutaneous dosing.

Key advances

- The use of triplet regimens substantially improves the duration of remission compared with doublet regimens in the treatment of both newly diagnosed[1] and relapsed multiple myeloma (MM)[2,3]
- In particular, the anti-CD38 antibody daratumumab has emerged as an important new treatment option for patients with relapsed MM, and can be combined effectively with existing therapies[2,3]
- Achieving very deep responses to treatment is now possible in patients with MM, necessitating the development of tools to assess minimal residual disease, and prompting revision of the response criteria[4]

In the CASTOR study[2], 498 patients with relapsed MM were randomly assigned to receive daratumumab, bortezomib, and dexamethasone (DVd), or bortezomib plus dexamethasone (Vd). The patients had received a median of two prior lines of therapy (range 1–10); about one-third were refractory to their previous treatment[2]. A prespecified interim analysis demonstrated substantially longer PFS and a higher ORR with the daratumumab-based triplet (TABLE 1). These outstanding results led the independent data and safety monitoring committee to recommend offering daratumumab monotherapy to the patients in the control arm upon disease progression.

The larger POLLUX trial[3] ($n = 569$) was published soon after CASTOR[2], and confirmed that daratumumab is a seminal development in MM therapy. In the CASTOR study[2], Vd was used in both arms for a fixed duration (± daratumumab), after which the trial essentially became a comparison of continuous daratumumab (in the DVd arm) versus no therapy (in the Vd arm). POLLUX[3] had a superior design that enabled testing of the continuous use of a daratumumab, lenalidomide, and dexamethasone (DRd) triplet regimen versus the Rd doublet in patients with relapsed MM. The results were striking, and removed any doubts about the substantial activity of daratumumab: use of DRd resulted in an unprecedented 63% reduction in the risk of progression or death compared with that associated with Rd therapy[3] (TABLE 1). An important finding from both CASTOR and POLLUX[2,3] was that a benefit of adding daratumumab to established doublets persisted irrespective of the number of prior lines of therapy — although, inarguably, greater benefit was noted when the triplet regimen was used earlier in the disease course. Another noteworthy finding of these two studies was that daratumumab-related infusion reactions occurred in 45–48% of patients, but >90% of these events occurred only upon the first infusion[2,3], indicating that repeated dosing is safe. On the basis of these studies, DRd (in patients not refractory to full-dose lenalidomide) and DVd (in those refractory to lenalidomide) are our preferred treatment options at first MM relapse (www.mSMART.org). Both regimens were approved in November 2016 by the FDA for the treatment of patients with MM who have received at least one prior therapy. An important unknown factor is the relative effectiveness of these triplets in patients previously treated for a prolonged period with lenalidomide as initial therapy — a growing population that was not well-represented in CASTOR and POLLUX[2,3].

With such substantive advances in MM therapy, updating of the 2008 International Myeloma Working Group (IMWG) response criteria to reflect the effectiveness of the newer combinations, and particularly to uniformly categorize the increased depth of response seen with these modern regimens, was inevitable[4]. The advanced platforms of next-generation sequencing (NGS) and next-generation flow cytometry enable the detection of one MM cell among 10^5–10^6 cells present in the bone-marrow compartment. Additionally, the use of PET–CT enables comprehensive assessment of complete eradication of both intramedullary and extramedullary disease. Acknowledging the promise of new analytical methods to detect minimal residual disease (MRD), in 2016, the IMWG issued revised response criteria that, among other changes, include new MRD categories to standardize reporting across clinical trials[4]. For a patient to be considered MRD-negative, they must have no phenotypically aberrant clonal plasma cells detectable in bone-marrow aspirates by either next-generation flow cytometry or NGS assays with a minimum sensitivity of ≥1 in 10^5 nucleated cells[4]. Two other MRD categories with additional requirements of normalization of PET–CT findings (imaging MRD-negative) and sustained negativity for at least 1 year (sustained MRD negative) were also defined[4]. These changes reflect the importance of MRD status as a key prognostic marker, but this information alone is insufficient: response-adaptive trials are needed to determine whether results of MRD testing can guide treatment decisions, such as adding new drugs for MRD-positive patients, or stopping or reducing the duration of therapy for MRD-negative patients.

The valuable information gleaned from these key studies in the past year has provided fresh perspectives and many moments of clarity in the increasingly complex field of MM therapy. For a patient diagnosed with MM in 2017, we now have treatments that can provide durable remissions both in the newly diagnosed and relapsed settings. Daratumumab might, in the future, be added to several existing treatment regimens in three-drug and four-drug combinations, and might soon be advanced to the frontline setting. We are very pleased with this progress, but also cognizant that much more needs to be achieved. The type of MM therapy we describe is unavailable in most parts of the world owing to regulatory and cost issues; this is simply unacceptable, as the survival benefits are striking[6,10]. Conspicuously absent from MM studies performed to date are data regarding incremental costs and quality-of-life implications related to managing MM with relentless therapy for many years. We have limited data on simultaneous, rather than the classic sequential, integration of newer drugs to the backbone regimens, as well as on the consequences of the frequent use of more-sensitive tools for response assessment. Finally, we need to continue to improve on outcomes because, as good as the results with novel regimens are, they still fall short of a cure. We are confident that ongoing trials in patients with MM are poised to move us in that direction, and anticipate the speed of progress to accelerate in the coming years.

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SMALL-CELL LUNG CANCER IN 2016

Shining light on novel targets and therapies

Charles M. Rudin and John T. Poirier

In 2016, the pace of biological insights into small-cell lung cancer (SCLC) was reflected in new treatment approaches that have suggested meaningful clinical benefit to patients. We focus on three highlights of 2016: preclinical studies defining NFIB as a putative driver of metastasis, and two clinical studies; one that assessed the efficacy of an agent targeting the Notch ligand DLL3, and the other that explored T-cell checkpoint-blockade therapies targeting PD-1 and CTLA-4.

Small-cell lung cancer (SCLC) has been an extremely frustrating cancer for the oncologist to tackle — although tantalizingly chemoresponsive, and seemingly at the cusp of cure, this aggressive tumour type is typified by rapid recurrence, extensive metastatic dissemination, and a consistently dismal prognosis. The clinical armamentarium for patients with SCLC has changed minimally over several decades. In the past few years, however, we have witnessed an accelerating pace of biological insights into the disease which, excitingly, began to bear fruit in 2016, with new treatment approaches showing meaningful clinical efficacy. Herein, we focus on three select highlights of 2016: preclinical studies defining the transcription factor NFIB as a putative driver of metastasis[1–3]; and two clinical studies, one that assessed targeting the Notch ligand, delta-like ligand 3 (DLL3)[4], and the other of drugs targeting the T-cell checkpoint receptors programmed cell-death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)[5].

A hallmark of SCLC is the predilection for early metastatic spread. Three studies[1–3] were published in short succession in 2016, in which the use of genetically engineered mouse models (GEMMs) of metastatic SCLC, driven by lung-specific Cre-mediated inactivation of *Trp53* and *Rb1,* helped to elucidate putative drivers of metastasis. In this model system, tumours spontaneously acquire additional genetic alterations that are associated with acceleration of tumour progression and metastasis. Recurrently observed alterations include loss of the tumour suppressor gene *Pten*, as well as copy number gain of the oncogenes *Mycl* and *Nfib*.

All three studies focused primarily on the role of *Nfib* in tumour progression and metastasis. Data from previous studies showed that spontaneous amplification of *Nfib* is a common secondary genetic alteration in this model, and that *Nfib* expression is associated with an elevated risk of metastasis in patients, although amplifications of this gene are rarely observed in human cancers[6]. All three groups found that *Nfib* drives a neural transcription programme that included genes regulating cell proliferation and migration. Semenova *et al.*[2] and Wu *et al.*[3] showed that spontaneous *Nfib* amplification was more common in metastases than in primary tumours, and both groups demonstrated that enforced expression of *Nfib* accelerated tumour growth and metastasis, while also broadening the spectrum of metastatic sites. Semenova *et al.*[2] further showed that while *Nfib* overexpression can cooperate with *Mycl* in accelerating tumour growth, the capacity to dramatically enhance metastasis and chromosomal instability was unique to *Nfib*.

Denny *et al.*[1] introduced a fluorescent Cre reporter into the *Trp53/Rb1* knockout mice to facilitate isolation of cancer cells from primary tumours and liver metastases at ultra-high purity. Strikingly, SCLC tumour samples clustered into two distinct chromatin accessibility states, the hyperaccessible state being associated with *Nfib* amplification and metastasis. Delving deeper, the researchers demonstrated that binding of Nfib to gene-distal regulatory regions of the genome maintains site-specific chromatin accessibility and seems to increase expression of nearby genes.

Together, these reports highlight the value of high-fidelity mouse models of cancer, especially in a disease context (metastatic SCLC) in which human samples are rare. At present, the therapeutic implications of these findings remains unclear; historically, transcription factors have typically been recalcitrant therapeutic targets. Further investigation into factors cooperating in the interaction between Nfib with chromatin, and a better understanding of how *Nfib* expression is induced and regulated is warranted to identify candidate therapeutic targets involved in disease progression and metastasis of human SCLC.

Inhibition of the Notch developmental pathway has been implicated in the development of neuroendocrine precursors in the lung and, more recently, in SCLC oncogenesis[7]. At the end of 2015, the inhibitory Notch ligand DLL3 was reported to be markedly upregulated and aberrantly expressed in high-grade neuroendocrine tumours, including SCLC[8]. The tumour-specific cell-surface expression of DLL3 provides a clear therapeutic opportunity. Preclinical testing of a novel antibody-drug conjugate with specificity for DLL3, rovalpituzumab tesirine (Rova-T), demonstrated impressive antitumour activity, and implicated DLL3 expression as a predictive biomarker for efficacy of this agent[8].

In 2016, results of a first-in-human phase I clinical trial of Rova-T were reported[4]. This trial enrolled a total of 74 patients with recurrent metastatic SCLC and eight patients with similarly recurrent large-cell neuroendocrine carcinoma of the lung. The study defined a recommended schedule of administration, and identified dose-limiting toxicities that included thrombocytopenia, liver function abnormalities, and serosal effusions. Among the patients with SCLC treated at active dose levels, an overall objective response rate of 18% was observed. Perhaps most notably, the preclinical suggestion of a predictive biomarker for this targeted therapy seemed to hold up: among the patients from whom tumour material was available for correlative analyses, only those with high levels of expression of DLL3

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Key advances

- Multiple groups studying genetically engineered mouse models have identified NFIB as a key driver of metastasis in small-cell lung cancer (SCLC)[1–3]
- Rovalpituzumab tesirine, an antibody-drug conjugate directed at the inhibitory Notch ligand DLL3, showed clinical activity in SCLC, with DLL3 protein expression being a potential predictive biomarker[4,8]
- T-cell checkpoint blockade using nivolumab, with or without ipilimumab, has demonstrated clinical activity in SCLC, regardless of PD-L1 expression on tumour cells[5]

experienced objective responses. Among all patients in this DLL3-high cohort (around two thirds of patients with SCLC) the confirmed objective response rate was 38% (10 of 26 patients), the disease control rate 88% (23 of 26 patients), and 1-year survival rate was 32%: these preliminary data are impressive in the context of recurrent metastatic SCLC. On this basis of these initial promising results, multiple confirmatory phase II studies of Rova-T have been launched this year.

No other area of clinical cancer research has generated more excitement than T-cell checkpoint blockade: monoclonal antibodies against PD-1, its cognate ligand PD-L1, and CTLA-4, have shown impressive activity in multiple malignancies. In 2016, the first studies evaluating these agents in SCLC were reported. Antonia *et al.*[5] published the results of a phase I–II trial of the anti-PD-1 antibody nivolumab, with or without the anti-CTLA-4 antibody ipilimumab, in patients with recurrent SCLC. This study included 216 patients, 98 of whom were treated with nivolumab alone, with almost all of the remaining patients receiving one of two combination regimens: nivolumab 3 mg/kg with ipilimumab 1 mg/kg every 3 weeks, or conversely, nivolumab 1 mg/kg with ipilimumab 3 mg/kg on the same schedule. In general, the toxicity spectra observed were similar to those reported in other studies of these agents in patients with cancer, including grade 3–4 treatment-related adverse events in 13% of patients treated with nivolumab and in 19–30% of patients in the combination arms.Nivolumab alone was associated with a 10% response rate and a 32% disease control rate, while the combination treatment cohorts had response rates of 19–23% and disease-control rates of 36–42%. The 1-year survival was 33% with nivolumab alone, and 35–43% in the combination arms. These impressive results led to incorporation of the nivolumab–ipilimumab combination regimen into the National Comprehensive Cancer Network treatment guidelines for SCLC, in 2016.

Many questions and opportunities in immunotherapy remain. In contrast to other cancers, PD-L1 positivity is rare in SCLCs, and does not seem to correlate with benefit from PD-1 directed therapy. The results of the study by Antonia *et al.*[5] indicate that ipilimumab might augment the activity of nivolumab, although a recent phase III study of ipilimumab with or without chemotherapy in SCLC was entirely negative, suggesting limited or no activity of CTLA-4 blockade in this context[9]. More encouragingly, in 2016, other more novel immunological targets, notably the macrophage 'don't-eat-me' signal CD47, emerged as promising new opportunities in the therapeutic landscape for SCLC[10].

SCLC remains a lethal disease, despite the recent progress in defining novel targets and therapeutic strategies. Multiple laboratory investigators are now focusing on critical aspects of SCLC biology, including the remarkably rapid shift from chemosensitive to chemoresistant disease. Several groups are generating increasingly complex and manipulable preclinical models of disease, which might further define drivers of oncogenesis and metastatic spread. Most notably, preclinical insights are informing clinical trials and thus, enabling the translation of scientific discoveries into clinical benefits. These parallel and integrated identification and validation efforts are shining new lights in what has been a dark corner of clinical oncology.

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HEAD AND NECK CANCER IN 2016

A watershed year for improvements in treatment?

Alain P. Algazi and Jennifer R. Grandis

In the past year, clinical trials have provided important information on strategies to decrease treatment-associated toxicities in patients with head and neck cancer. In addition, the FDA approved the first immunotherapeutic agents for patients with recurrent and/or metastatic disease, based on the observation of durable responses to pembrolizumab in a phase Ib trial, and demonstration of improved survival and quality of life with the use of nivolumab versus chemotherapy in a phase III trial.

The management and prognosis of patients with squamous-cell carcinoma of the head and neck (SCCHN) is generally dependent on the stage of disease, and standard treatments are associated with considerably morbidity. Treatment of early stage disease with curative intent using surgery, or radiation-based approaches results in a high probability of long-term survival. For patients with metastatic disease, however, effective treatment

Key advances

- PET–CT imaging enables detection of residual disease after chemoradiotherapy (CRT) in patients with squamous-cell carcinoma of the head and neck (SCCHN) and extensive nodal disease; surgery can be avoided in those without detectable residual disease[1]
- Studies[3,4] have provided additional data on the potential applications and limitations of anti-EGFR therapy with cetuximab in patients undergoing definitive CRT
- The anti-PD-1 antibodies pembrolizumab and nivolumab have now been approved by the FDA for the treatment of patients with platinum-refractory recurrent and/or metastatic SCCHN, based on data from a phase Ib study[7,8] and a phase III trial[6], respectively
- In the phase III trial[6] of nivolumab, use of this agent was associated with improved overall survival and quality of life, compared with the use of standard-of-care chemotherapy

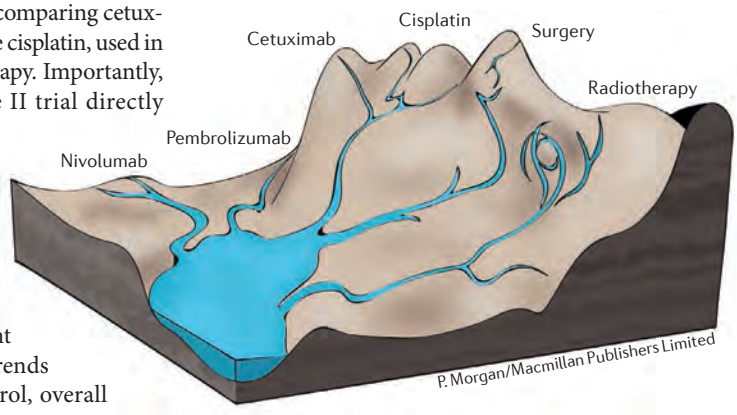
options have been limited, with the most-aggressive medical therapy yielding a median overall survival of only 10 months. Moreover, even patients with early stage disease often experience life-altering adverse effects following definitive therapy. In the past year, new data have challenged some of the fundamental assumptions about treatment strategies and treatment-associated toxicities in patients with SCCHN. These data have come from studies focused on decreasing treatment-related morbidity through the judicious use of surgery, radiation therapy, and cytotoxic chemotherapy. Novel findings also support an expanded role for monoclonal antibodies in both the definitive and metastatic treatment settings, particularly the anti-PD-1 antibodies nivolumab and pembrolizumab, which confer durable remissions in some patients, as observed across a wide spectrum of other metastatic cancer types.

For patients with SCCHN, disfigurement and functional losses following surgical resections provided the rationale for the development of chemoradiotherapy (CRT) approaches: a complete response to CRT can completely eliminate the need for surgery in certain individuals, and in those with an incomplete response, the extent of resection can be substantially reduced. Previous retrospective analyses have suggested that negative PET–CT imaging findings after definitive CRT indicate a complete treatment response, but these observations had never been validated prospectively. In a phase III non-inferiority trial[1] with results reported in 2016, 564 patients with SCCHN and extensive nodal involvement (N2–3) were randomly assigned to CRT followed by either planned neck dissection within 4–8 weeks, or PET–CT imaging at 12 weeks; in the PET–CT cohort, only those with an incomplete or equivocal response proceeded to surgery. At 2 years, no statistically significant differences in overall survival or locoregional control were demonstrated between the two groups[1]. Furthermore, fewer surgeries (54 versus 221) and, thus, fewer surgical complications were reported for the PET–CT group — the rates of surgical complications among those who underwent surgery were similar in both groups, as would be expected[1]. Our ability to generalize these findings to all patients with SCCHN might be limited, however, because ~85% of study participants had oropharyngeal carcinoma, most of whom had human papillomavirus (HPV)-positive disease[1]; HPV-positive tumours are known to portend a more-favourable prognosis than HPV-negative disease.

Platinum-based chemotherapy combined with radiotherapy can cure patients with locally advanced SCCHN, but such treatment is a major cause of morbidity, including permanent renal dysfunction, hearing loss, and neuropathy. Since 2006, treatment with the anti-EGFR antibody cetuximab has been regarded as a potentially less-toxic alternative to platinum-based chemotherapy, after the results of the pivotal phase III IMCL-9815 trial[2] demonstrated improved outcomes in patients treated with definitive radiotherapy and cetuximab versus radiotherapy alone. Subgroup analyses have suggested that the benefits of cetuximab are restricted to patients with oropharyngeal carcinoma, perhaps owing to the strong association of these tumours with HPV infection. In the past year, however, a retrospective analysis involving 182 patients with locally advanced oropharyngeal carcinoma enrolled in the IMCL-9815 trial demonstrated that the benefits of cetuximab were not limited to patients with HPV-positive tumours, although this group had better overall survival than those with HPV-negative tumours[3]. Nevertheless, no prospective data were available from studies comparing cetuximab versus standard-of-care cisplatin, used in combination with radiotherapy. Importantly, in 2016, results of a phase II trial directly comparing cetuximab versus weekly cisplatin as a radiosensitizer in patients with oropharyngeal cancer were reported[4]; the data demonstrated differences in acute toxicities between the treatment arms, and nonsignificant trends towards inferior local control, overall survival, and disease-specific survival in the patients who received cetuximab[4]. Of note, only 70 of the 130 planned patients were enrolled in this non-inferiority trial owing to slow accrual. A larger phase III trial comparing these two radiosensitizers, RTOG-1016 (NCT01302834), has completed enrolment, and the results are eagerly awaited.

The adverse effects of definitive radiotherapy are also a major source of morbidity and, given the favourable prognosis of patients with HPV-positive oropharyngeal carcinoma, several studies are exploring whether decreased doses of radiation are effective in this population. In a phase II trial[5], with results published in December 2015, 37 of 43 patients (86%) had a pathological complete response to reduced-intensity radiotherapy (maximum total dose of 60 Gy) and concurrent cisplatin — standard radiotherapy regimens use a total dose of 66–74 Gy. A randomized phase II trial of reduced-intensity radiotherapy either with or without concurrent cisplatin chemotherapy that will include 296 patients is ongoing (NCT02254278), and should provide more data on the potential for radiation-dose de-escalation in patients with advanced-stage, HPV-associated oropharyngeal carcinoma.

The most clinically significant developments for patients with head and neck cancer over the past year inevitably relate to anti-PD-1 immunotherapy, with the FDA approval of nivolumab and pembrolizumab for the treatment of platinum-refractory recurrent and/or metastatic disease. In a phase III trial[6], nivolumab monotherapy induced objective tumour responses in a modest 13.3% of patients, compared with a response rate of 5.8% in patients who received standard-of-care methotrexate, docetaxel, or cetuximab. The increased response rate with nivolumab translated into a 2-month increase in median overall survival[6]. Importantly, the nivolumab cohort had a 20% lower incidence of grade 3–4 toxicities and better quality-of-life outcomes than those of the standard-of-care cohort[6].



These data led to the approval of nivolumab in November 2016. Pembrolizumab had been approved by the FDA in August 2016, based on data from a nonrandomized phase Ib trial[7,8] demonstrating an overall response rate of 18% and tumour volume reductions in about half of the 132 patients with platinum-refractory recurrent and/or metastatic SCCHN. These results are indicative of modest improvements over the previous standard of care, but findings in other solid-cancer settings indicate that anti-PD-1 antibodies enhance immune memory, leading to long-term remissions in some patients. The data suggest the same effects are possible in patients with SCCHN, with 1-year survival of 36% with nivolumab versus 16.6% with standard-of-care therapy[6]. Moreover, in the studies of nivolumab and pembrolizumab[6–8], a substantial minority of patients had ongoing responses at the time of data analysis.

Patient selection and combination therapy based on pretreament tumour characteristics might substantially increase the clinical utility of anti-PD-1 antibodies in the treatment of SCCHN. Binding of PD-L1 to PD-1 on cytotoxic T cells results in suppression of T-cell proliferation and cytokine production. Correspondingly, high levels of intratumoural PD-L1 expression have been associated with increased responsiveness to anti-PD-1 antibodies. In the phase III study of nivolumab[6], subgroup analyses revealed that the survival benefit was mostly restricted to patients with ≥1% intratumoural PD-L1 expression (57.3% of patients with evaluable tumour specimens). In this PD-L1-positive population, the median overall survival duration of patients treated with nivolumab was nearly double that of patients who received chemotherapy (8.7 months versus 4.6 months); the median overall survival of patients with <1% intratumoural PD-L1 expression was ~5.7 months, independent of the treatment they received. Additional assays have shown promise in identifying patients who are mostly likely to benefit from anti-PD-1 monotherapy. In particular, gene-expression profiling has revealed an 'interferon-γ' signature that is highly correlated with overall response rate, progression-free survival, and overall survival in patients with SCCHN treated with pembrolizumab[9]. Baseline tumour characteristics, such as the degree of intratumoural lymphocyte infiltration or the presence of immunosuppressive regulatory T (T_{reg}) cells, might also help to select specific therapeutic combinations that have been demonstrated to target specific immunological deficiencies. For example, use of anti-CTLA-4 antibodies in combination with anti-PD-1 antibodies might increase tumour lymphocyte infiltration, and the addition of an indoleamine 2,3-dioxygenase inhibitor might decrease the relative proportion of T_{reg} cells.

Many of the advances in the treatment of patients with head and neck cancer over the past year seem modest at a population level, but several key principles that are likely to improve the outcomes of these patients have been embraced. Decreasing the incidence and/or severity of treatment-associated toxicities for patients with curative options has been a major area of interest and the rational development of immunotherapy raises the prospect of long-term remissions, even in patients with unresectable or metastatic SCCHN. If we can expand on these efforts in the future, 2016 might prove to have been a watershed year.

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SARCOMA IN 2016

Evolving biological understanding and treatment of sarcomas

Jean-Yves Blay and Isabelle Ray-Coquard

In 2016, novel findings on the role of predisposing gene variants in sarcoma oncogenesis were published, as well as studies addressing novel molecular classifications and results from randomized controlled trials highlighting successful new treatments. Herein, we discuss these meaningful advances.

Soft-tissue and visceral sarcomas are a group of very rare cancers arising from resident cells of the connective tissues. The overall incidence of these malignancies (comprising >80 histological subtypes and an even larger number of molecular subtypes) is close to 5 per 100,000 per year[1]. In comparison with carcinomas, sarcomas predominantly affect children (15% of paediatric cancers) and young adults (<25 years of age). The mechanisms of sarcoma oncogenesis remain largely unclear, with the exception of rare predisposition syndromes that have been identified, such as the Li–Fraumeni syndrome. Ballinger and co-workers investigated the constitutional genetic landscape of bone and soft-tissue sarcomas in routine clinical practice[2]. In this study, genetic analysis was performed in 1,162 patients (aged 29–60) with a histologically confirmed diagnosis of sarcoma and in 6,545 healthy individuals using targeted exon sequencing of 72 genes selected on the basis of associations with increased cancer risk[2]. Rare variants were stratified into classes that matched the WHO classification of genetic variation, with classes 5 to 3 representing, respectively, variants with known, expected, or predicted pathogenic signification[3]. A case-control analysis of the

Table 1 | **Results from randomized trials in advanced-stage sarcoma published in 2016**

Study	Histotypes	Agents compared	Median OS	Median PFS
Tap *et al.*[8]	All	Doxorubicin versus olaratumab + doxorubicin	14.7 months versus 26.5 months; $P = 0.0003$	4.1 months versus 6.6 months; $P = 0.06$
Demetri *et al.*[9]	LPS and LMS	Dacarbazine versus trabectedin	12.4 months versus 12.9 months; $P = 0.38$	1.5 months versus 4.2 months; $P > 0.001$
Schoffski *et al.*[10]	LPS and LMS	Dacarbazine versus eribulin	11.5 months versus 13.5 months; $P = 0.01$	2.6 months (both); $P = 0.23$
Penel *et al.*[11]	Non-LPS	Placebo versus regorafenib	1.0 months versus 4.0 months; $P < 0.0001$	9.5 months versus 13.4 months; $P = 0.06$
	LPS	Placebo versus regorafenib	1.7 months versus 1.1 months; $P = 0.70$	8.8 months versus 4.7 months; $P = 0.21$

LPS, liposarcoma; LMS, leiomyosarcoma; OS, overall survival; PFS, progression-free survival.

rare variant burden in patients with sarcoma and their relatives versus a reference population was performed, and 638 of 1,162 patients with sarcoma were found to have a total of 956 class 3–5 rare pathogenic germ-line variants. Of these, variants of genes frequently mutated in sarcoma (*TP53, TSC2, SDHB, RB1* or *PTEN*), but also variants of other genes (*ATM, ATR, BRCA2* or *ERCC2),* were found to be associated with an increased risk of sarcoma. Individuals with pathogenic variants in classes 5–3 had a predisposition to being diagnosed with sarcoma at an earlier age than those with other forms of sarcoma. Among the families tested, 17% met the criteria of a genetic predisposition to cancer syndrome, and 16% only had a known or expected pathogenic variant. Conversely, only 20% of patients carrying these variants were found to have a cognate syndrome. Moreover, for 240 patients harbouring multiple (2–6) pathogenic variants of any classes, the number of pathogenic variants was inversely correlated with age at diagnosis, suggesting a polygenic contribution. The results of this study demonstrated the presence of putative pathogenic monogenic and/or polygenic variation in 50% of patients with sarcoma, shedding new light on the aetiology of this disease and suggesting possible management strategies for individuals at risk of developing sarcoma.

The first-line treatment options for patients with advanced-stage soft-tissue sarcoma (ASTS) have not progressed substantially in the past 30 years. The results of EORTC 62012, the largest ever phase III trial addressing this question[4] showed that the combination of optimal doses of ifosfamide and doxorubicin significantly improved progression-free survival (PFS) duration in patients with ASTS, but had no significant effects on overall survival. Similarly, neither the combination of gemcitabine with docetaxel[5], doxorubicin with palifosfamide[6], nor doxorubicin with evofosfamide[7] led to improvements in PFS or overall survival durations over doxorubicin alone in the GEDDIS[5], PICASSO III[6] and NCT01440088 (REF. 7) trials, respectively. In this context, the results of the randomized phase II study reported by Tap *et al.*[8] that assessed the addition of olaratumab, a monoclonal anti-PDGFRα antibody, to a doxorubicin-based regimen are a remarkable advance. The median overall survival duration was improved by 11.8 months, and the primary end point of a 2.5-month improvement in median PFS duration for the combination group was also met (TABLE 1). The response rates were not significantly different between groups, and slightly more toxicities were observed among patients in the combination arm — in particular in terms of the incidence of haematological toxicities and mucositis. The results of this study[8] led to the approval of this combination by the FDA and a positive recommendation by the European Medicines Agency. A phase III study testing olaratumab plus doxorubicin has been completed (NCT02451943); if this trial is confirmative, this combination might become the standard first-line treatment option for patients with ASTS. Of note, the response rate of patients receiving doxorubicin with olaratumab remained inferior to that reported in those trials receiving doxorubicin plus ifosfamide[4] and thus, the clinical validity of combining ifosfamide, doxorubicin and olaratumab will need to be addressed.

The approved agents for patients with progressive disease after doxorubicin treatment include dacarbazine, trabectedin and pazopanib. Other cytotoxic agents have shown efficacy in phase II studies, but have not been approved (such as paclitaxel for patients with angiosarcoma, or gemcitabine with docetaxel or dacarbazine, in leiomyosarcoma and other subtypes). The results of a phase III study[9] demonstrated that trabectedin improves PFS duration over dacarbazine in patients with metastatic leiomyosarcoma or liposarcoma resistant to doxorubicin, but was not accompanied by a significant improvement in overall survival duration (TABLE 1). Schoffski *et al.*[10] reported the results of a phase III study comparing eribulin with dacarbazine in patients with metastatic leiomyosarcoma or liposarcoma resistant to doxorubicin. The median overall survival duration was significantly improved by 2 months, but no significant differences in PFS were observed (TABLE 1). Subgroup analysis showed an improvement of overall survival only in patients with liposarcoma (one third of the cohort). Together, these two trials with intriguing results and performed in similar patient populations[9,10] show that PFS is not a consistently reliable surrogate for overall survival.

To date, only one antiangiogenic molecule, pazopanib, has been approved for the treatment of patients with ASTS (not liposarcoma) with progressive disease after receiving doxorubicin. Antiangiogenic agents have been tested in a few other randomized clinical trials for patients with sarcoma (for example, in NCT01303497, bevacizumab was tested in patients with angiosarcoma with negative results). In RegoSARC[11], a randomized phase II study, regorafenib was compared with placebo in patients with ASTS with disease progression after doxorubicin. Four different subgroups were analyzed: patients with liposarcoma, leiomyosarcoma, synovial sarcoma, or other sarcomas. Regorafenib improved PFS duration, and, marginally, overall survival duration in all patients except those with liposarcoma[11]. Importantly, most of the patients included had not been exposed to pazopanib. Thus, the efficacy of regorafenib in patients with ASTS (excluding liposarcoma) previously treated with pazopanib is now being tested in a fifth treatment arm (NCT01900743). Two other ongoing trials are exploring the efficacy of regorafenib in patients with bone sarcoma (NCT02389244, NCT02048371).

The identification of specific molecular subtypes within a single histological entity,

Key advances

- Outside well-defined cancer-predisposition syndromes, sarcomas occur preferentially in individuals with one of several pathogenic variants of cancer-associated genes[2]
- Newly defined regimens containing olaratumab and doxorubicin with eribulin or regorafenib were successfully tested in several groups of patients with sarcoma[8–11]
- Most wild-type gastrointestinal stromal tumours are *SDH*-inactivated, more likely by mutations but also by methylation[12]

such as gastrointestinal stromal tumours (GIST), can provide insights into the specific natural history of such variants. Given the rarity of these subtypes, worldwide collaborations are often needed to obtain sufficiently large patient populations to provide statistically robust data. Most patients with wild-type GISTs (that is, with no mutations in *KIT* nor in *PDGFRA*) are usually children or young adults. Boikos *et al.*[12] described the molecular heterogeneity and natural history of these very rare wild-type GISTs. In their study of 95 evaluable patients, three subgroups were identified according to the status of *SDH* (encoding succinate dehydrogenase): *SDH*-competent GIST (11%), *SDH*-deficient GIST owing to gene mutations (*SDH*-mutant; 67%), or caused by methylation (*SDH*-epimutant; 22%). Interestingly, germ-line *SDH* mutations were also present in 82% of the patients, a proportion markedly higher than that found in those with GISTs harbouring *KIT* or *PDGFRA* mutations. *SDH*-competent tumours had mutations in *NF1*, *BRAF*, or *ARID1A*. Differences exist between the clinical presentation of *SDH*-deficient and *SDH*-competent GISTs: the former occur only in the stomach, are often metastatic at diagnosis (lymph-node metastasis), and tend to have an indolent course despite limited responses to imatinib (2% of patients) or sunitinib (19% of patients). In the *SDH*-deficient group, 21% of patients had syndromic GISTs (Carney triad syndrome or Carney–Stratakis syndrome). *SDH*-competent GISTs occur mostly in the small bowel, and are rarely metastastic. To summarize, this study described the molecular heterogeneity and distinct natural history of three molecular subgroups within wild-type GIST, illustrating the need and efficacy of performing worldwide collaborative studies to explore very rare forms of sarcomas. In the years to come, such collaborative studies will be critical to improve the outcomes of patients with sarcoma.

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NEUROENDOCRINE TUMOURS IN 2016

Defining rules for increasingly personalized treatments

Massimo Falconi and Stefano Partelli

S.Bradbrook/NPG

In 2016, results of an extensive trial broadened the range of malignancies that can be treated with everolimus to include neuroendocrine tumours (NETs) of the lung and gastrointestinal tract. Furthermore, studies aimed at identifying biomarkers with increased specificity, and at better defining high-grade NETs have enabled substantial progress towards delivering effective targeted treatments to patients with NETs.

In the past decade, substantial advancements in neuroendocrine tumour (NET) research have occurred. As a result, several therapies with proven efficacy are now available for patients with NETs, but many questions remain unanswered, including the clinical criteria for selection of patients who are most likely to benefit from targeted therapies, the optimal sequence of treatments, and the optimal potential biomarkers indicative of treatment efficacy. Neuroendocrine neoplasms are traditionally regarded as a single group of malignancies; however, NETs can be subcategorized according to different features of their clinical presentation, and, more importantly, by their distinct site of origin — a feature frequently used to assign a prognosis. On the basis of these distinctions, patients can be assigned to participate in specific clinical trials in which they receive tailored treatment that is concordant with the site of the primary tumour.

In 2016, results from a large, international, randomized clinical trial (RADIANT-4)[1] provided evidence for extending the use of everolimus to include progressive NETs of the lung and gastrointestinal tract. Previously, everolimus had been demonstrated to prolong progression-free survival (PFS) duration in patients with advanced-stage pancreatic NETs when compared with placebo[2]. Subsequently, a growing number of experts proposed that this drug could be used to treat patients with NETs

in other organs. The results of RADIANT-4 confirmed this hypothesis, revealing a consistent treatment benefit across patients with NETs with different primary tumour sites[1]. In this study, the estimated PFS at 12 months was 44% for patients in the everolimus group compared with 28% in the placebo group. Moreover, this retrospective study[1] showed consistent beneficial effects on PFS across the entire spectrum of the subgroups, defined on the basis of primary tumour origin (lung, gastrointestinal or unknown). Before being enrolled in the RADIANT-4 trial, patients were categorized into prognostic groups; only those with low-grade or moderate-grade lesions (G1 or G2) were selected for inclusion. Since the publication of the 2010 WHO classification of NETs[3], oncological trials have primarily focused on patients with these G1 or G2 forms of the disease, strictly adhering to the practice of using pretreatment Ki67 index scores as the main criterion for selecting eligible patients. Consequently, we have witnessed an increased availability of therapeutic options for patients with G1 or G2 NETs, whereas the need for effective new treatments for patients with high-grade (G3) NETs remains largely unmet.

This year, the results of a breakthrough study[4] conducted at the Memorial Sloan Kettering Cancer Center (MSKCC; New York, USA) were published, providing strong evidence to support the unique distinction of well-differentiated NETs (WD–NETs) with a high-grade component (characterized by well-differentiated histopathological features and a low proliferative rate) as a separate entity within high-grade NETs. The analysis of survival data strongly supported distinction on the basis of this specific feature; patients with WD–NETs with a high-grade component had a median disease-specific survival (DSS) duration of 55 months, whereas median DSS duration was 16 months for patients with poorly differentiated G3 neuroendocrine carcinomas (PD–NECs) without a well-differentiated component. These findings do not merely imply that a pathological reclassification of high-grade NETs into WD–NETs and PD–NECs is needed, but they also have substantial implications for treatment guidelines. In particular, patients with PD–NEC usually benefit from cisplatin and/or etoposide-based regimens, whereas the combination of streptozocin and 5-fluorouracil seems to be effective in patients with WD–NETs. This study has enabled the distinction between two different categories of high-grade neoplasms, the most aggressive category within NETs; such insights can lead to a broadening of the scope of available treatments for patients with these malignancies, at least in relation to future clinical trials. The challenge of obtaining a definitive diagnosis of WD–NET with a high-grade component versus that of PD–NECs remains, however, and is heightened by further diagnostic obstacles, such as those related to the amount of tissue available for diagnosis which, for patients with advanced-stage tumours, typically means biopsy samples rather than surgical specimens.

In 2016, another study[5] from the same team at MSKCC honed in on this diagnostic issue, and set forth a simple and comprehensive set of guidelines to differentiate WD–NETs with a high-grade component from PD–NECs. A notable methodological advance of this study was the development of a very practical approach for diagnosing this newly recognized subgroup[4], which was established through the meticulous evaluation of available specimens from previous clinical studies for specific biomarkers (such as DAXX, ATRX, Rb or p53, which are products of genes altered in patients with WD–NET). Using this approach, patients with WD–NETs with a high-grade component are now more likely to be accurately diagnosed than they were in the past using methods relying on the evaluation of only the morphological features and Ki67 index of the tumour. The morphological distinction of high-grade NETs will result in increased precision in patient categorization and will enable the delivery of individually tailored treatments.

Currently, somatostatin receptors are one of the few identified therapeutic targets in patients with NETs for which therapies are available, namely somatostatin analogues or peptide receptor radionuclide therapy (PRRT). NETTER-1 (REF. 6), a large prospective, international phase III trial, was designed to evaluate the role of the radiolabelled somatostatin receptor agonist ^{177}Lu-DOTA0-Tyr^3-octreotate (DOTATATE), compared with the nonlabelled agonist long-acting repeatable (LAR) octreotide, in the treatment of patients affected by advanced-stage NETs of the small bowel. The results demonstrated a significant increase in the median PFS duration of patients with midgut NETs who received DOTATATE compared with that of those treated with LAR octreotide (not reached versus 8.4 months; $P<0.0001$). This trial succeeded in establishing an additional effective therapeutic agent against these tumours, although the absence of accurate biomarkers to define therapeutic efficacy remains a substantial unmet clinical need.

To date, various blood-based biomarkers (in particular, chromogranin A) have been proposed, but none have exhibited sufficient accuracy for the prediction of tumour response to treatment. In light of this challenge, encouraging results have been obtained with the analysis of circulating gene transcripts before and after treatment in patients with NETs. This year, the levels of circulating NET transcripts (assessed using the 'NETest') and chromogranin A were used in a study[7] to evaluate the efficacy of PRRT with DOTATATE for the treatment of patients with NETs. Changes in NETest accurately correlated with a response to treatment (observed in 89% of patients with a response), whereas increases in chromogranin A levels were observed in only 24% of patients with a response. In the same study[7], a gene inference methodology was developed to identify specific 'omics' features (growth factor signalome and tumour metabolome) relevant to tumour behaviour. Expression of signalling (*ARAF1, BRAF, KRAS* and *RAF1*) and metabolic genes (*ATP6V1H, OAZ2, PANK2* and *PLD3*) detected in circulating tumour material before initiation of PRRT revealed 76% accuracy in predicting the subsequent treatment response; the accuracy increased to 94% when omics-derived information was combined with information on tumour grade. Notably, most patients with tumours exhibiting substantially elevated signalome and/or metabolome gene expression at baseline responded to PRRT (97%). The main implications of these observations are that therapeutic efficacy can be monitored via evaluation of defined markers and, in addition, the response to PRRT can be predicted through the identification of specific gene clusters.

Finally, data from another study published in 2016 (REF. 8) endeavoured to assess novel biomarkers of response to treatment. This study, conducted by a team based at the University College London Cancer Institute (London, UK), found that the quantification of circulating tumour cells (CTCs) might be a promising biomarker for evaluation of clinical outcome following treatment. In this study, researchers found that among patients

Key advances

- Results of a randomized clinical trial have confirmed that everolimus is associated with a marked improvement in progression-free survival duration in patients with neuroendocrine tumours (NETs) of the lung or gastrointestinal tract[1]
- Well-differentiated NETs with a high-grade component have been recognized as a unique entity within high-grade NETs; practical guidelines for classification of these entities have been described[4,5]
- The role of circulating gene transcripts and circulating tumour cells as possible biomarkers of treatment efficacy has been evaluated in patients with NETs[6–8]

with metastatic NETs, CTC counts at baseline were strongly indicative of the likelihood of extended overall survival. Specifically, a count of >8 CTCs in 10 ml of blood was associated with a median overall survival duration of 10.7 months, whereas a count of 1–8 CTCs in 10 ml of blood was associated with a median overall survival duration of 31.2 months; the median overall survival duration of patients with no detected CTCs was not reached at the time of analysis. These preliminary results are encouraging; however, for these potential novel biomarkers (NETest, 'omics' signatures and CTCs) to be adopted in daily clinical practice, both the reproducibility and the costs of these diagnostic procedures need to be taken into account. In 2017, we expect the therapeutic role of PRRT to be better clarified by the results from the NETTER-1 trial[6]. In addition, the optimal sequencing of treatments, which remains a major unmet clinical need in the management of patients with NETs, will be better investigated in the near future.

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RENAL-CELL CARCINOMA IN 2016

Advances in treatment — jostling for pole position

Laurence Albiges and Toni K. Choueiri

In 2016, two major trials provided conflicting evidence regarding the role of 1 year of adjuvant therapy with sunitinib for patients with high-risk renal-cell carcinoma. In the second-line metastatic setting, updated data from key trials showed that cabozantinib improved overall survival over everolimus, and nivolumab was associated with a better quality of life compared with everolimus. Finally, a phase II study in previously untreated patients showed cabozantinib to be superior to sunitinib.

Metastatic renal-cell carcinoma (RCC) has been notoriously resistant to systemic therapy, with only cytokines, such as IL-2 and interferon, producing rare remissions in a small subset of patients. Insights into the importance of targeting angiogenic pathways in RCC led to the development of an armamentarium of therapies over the past 10 years that are now considered 'standard'. Nevetheless, after a decade of treatment with VEGF and mTOR inhibitors, the metastatic RCC therapeutic landscape was deeply shaken in 2015, by the advent of three new active agents. Nivolumab demonstrated an overall survival benefit compared with everolimus — a standard of care — in the second-line setting[1]. A phase III trial showed that cabozantinib improved both progression-free survival (PFS) and response rate compared with everolimus[2]. Moreover, the combination of everolimus with lenvatinib, a dual VEGFR and FGFR inhibitor, was demonstrated to be efficacious in a randomized phase II study[3].

So what did 2016 bring? Since the late 2000s, several large adjuvant trials have been conducted in patients with RCC. Among these, two trials were published in 2016 and represent major steps forward in the exploration of the role of the VEGF axis in the adjuvant setting. The ASSURE trial[4] (ECOG – ACRIN E2805) was a double-blind, placebo-controlled, randomized, phase III trial that was conducted in 226 centres in North America. In total, 1,943 patients at high risk of disease recurrence were randomly assigned to receive sunitinib ($n = 647$), sorafenib ($n = 649$), or placebo ($n = 647$) for 1 year after surgery. The primary objective was to compare disease-free survival (DFS) between each experimental group and the placebo arm. The primary analysis showed no significant differences in DFS or overall survival between the three arms. Moreover, toxicity necessitated treatment discontinuation in >44% of patients in both experimental arms, subsequently leading to a protocol amendment to reduce the starting dose[4].

In the randomized, double-blind, phase III S–TRAC trial[5] (NCT00375674), 615 patients with locoregional, high-risk clear-cell RCC were assigned to receive either adjuvant sunitinib or placebo for 1 year; the primary end point was DFS, according to blinded independent central review. The median DFS was 6.8 years (95% confidence interval (CI) 5.8–not reached) in the sunitinib group and 5.6 years (95% CI 3.8–6.6) in the placebo group (HR 0.76; 95% CI 0.59–0.98; $P = 0.03$). In the sunitinib arm, dose reduction or treatment discontinuation was required owing to adverse events in 34.3% and 28.1% of patients, respectively[5].

Key advances

- In the adjuvant setting, for localized, high-risk clear-cell renal-cell carcinoma (ccRCC), 1 year of sunitinib improved disease-free survival compared with placebo in one trial, but not in another trial[4,5]
- In the second-line and third-line settings in patients with metastatic RCC, cabozantinib improved overall survival compared with everolimus[6], and new data suggests nivolumab is associated with quality-of-life improvement compared with everolimus[7]
- One randomized phase II trial showed cabozantinib to be superior to sunitinib in untreated patients with intermediate-risk or poor-risk metastatic ccRCC; in those with metastatic non-clear-cell RCC, sunitinib might have a slight edge over everolimus[8]

Stuart Gray/Alamy Stock Photo

The results of these two trials in patients with high-risk RCC differ completely and raise the following issues: first, patient-population selection; second, treatment exposure; third, end-point assessment. While ASSURE[4] did include a population of patients with non-clear-cell RCC (nccRCC) and a population at a lower risk of relapse, the researchers reported a prespecified subset analysis restricted to the clear-cell RCC (ccRCC) T3–T4 population only, and no differences were associated with either sunitinib or sorafenib treatment in this group.

The potential outcome discrepancy can also be attributed to difference in drug exposure, as approximately a third of patients in the ASSURE trial received sunitinib therapy at a one-level reduced starting dose[4], whereas in the S–TRAC trial[5], all patients started at a 50 mg dose. Another major differences between the two trials was related to the central radiological review for S-TRAC versus investigator assessment only in the ASSURE trial. Of note, in S-TRAC[5], the investigator assessment of the primary end point did not reach statistical significance.

In the light of adverse effects and absence of an overall survival benefit, patient adherence is a key consideration, and will necessitate individual counselling. Results of further adjuvant studies are eagerly awaited; upcoming studies will report on the role of pazopanib (PROTECT; NCT01235962), or everolimus (EVEREST; NCT01120249). The duration of adjuvant therapy — that is, 1 year versus 3 years of sorafenib, versus 3 years of placebo — is being investigated in the SORCE trial (NCT00492258). In the adjuvant trial of axitinib (ATLAS; NCT01599754) 3 years of axitinib compared with placebo will be assessed.

In the metastatic setting, 2016 had been a rich year, with the reporting of interim results from two pivotal studies of nivolumab and cabozantinib in the second-line setting, after failure of prior VEGFR-targeted therapies. The final overall survival data from the METEOR study[6] of cabozantinib in 658 patients with metastatic ccRCC were subsequently presented and reported in 2016; median overall survival was 21.4 months with cabozantinib and 16.5 months with everolimus (HR 0.66; 95% CI 0.53–0.83; $P=0.00026$). On the basis of these results, cabozantinib 60 mg per day was approved by the FDA and the European Medicines Agency in 2016, and has been integrated in international guidelines on the second-line treatment of patients with RCC who are unresponsive to previous treatment with one or more VEGFR tyrosine-kinase inhibitors. Since these trials were reported, selecting patients for nivolumab or cabozantinib therapy has become an important challenge: evidence supporting their use comes from phase III trials, but unfortunately the strategies have not been compared in a head-to-head fashion. Furthermore, both drugs exhibited benefit across all the prespecified subgroups (stratified, for example, by risk group, performance status, and number of prior lines of therapy). Cabozantinib had a noticeable benefit in patients with bones and visceral metastasis, as well as a low incidence of disease progression as a best response[6], suggesting a low incidence of primary resistance to this agent. On the other hand, nivolumab was associated with a quality-of-life improvement in a 2016 update from Checkmate 025, in addition to the known overall survival benefit compared with everolimus[7]. Finally, in both the Checkmate 025 (REF. 1) and METEOR trials[6], the attempt to use PD-L1 or MET staining as predictive biomarkers, respectively, failed to discriminate the patients more likely to benefit from the experimental drug.

In the randomized phase II CABOSUN trial[8], cabozantinib was compared with the current standard-of-care sunitinib in previously untreated patients with intermediate-risk or high-risk metastatic ccRCC. Data reported in 2016 demonstated that treatment with cabozantinib was associated with significantly longer PFS (8.2 months versus 5.6 months; $P=0.012$) and a higher objective response rate (46% versus 18%) compared with sunitinib, with similar rates of adverse events and treatment discontinuation. Despite the small size of this study, cabozantinib might become a first-line therapeutic option for patients with metastatic ccRCC. Final overall survival data are still maturing.

The rare nccRCC population remains a clinical challenge given the heterogeneity of the tumours this definition encompasses and the more limited benefit derived from targeted therapies compared with that observed for patients with ccRCC. In 2016, the results of ASPEN[9], a randomized phase II trial to compare first-line everolimus with sunitinib in patients with metastatic nccRCC, were reported. Among the 108 patients enrolled, sunitinib increased PFS compared with that achieved with everolimus, and the results were statistically significant based on preplanned statistical analyses (8.3 months versus 5.6 months; HR 1.41, $P=0.16$. The P value target of significance was <0.2). The results of two previous trials, ESPN[10] and RECORD-3 (REF. 11) had indicated that everolimus is not superior to sunitinib in the nccRCC subpopulation. Taken together, these data suggest that sunitinib might have a slight advantage over everolimus in the first-line setting of metastatic nccRCC, although data from confirmatory phase III trials are required.

Overall, 2016 kept the pace with 2015 in terms of the rapidly evolving RCC therapeutic landscape. Adjuvant use of sunitinib remains controversial and is not yet recommended in current guidelines[12]. Nivolumab and cabozantinib are new standards in the second-line setting, but cabozantinib might become the treatment of choice in the first-line setting based on data from CABOSUN[8]. Effective therapy in the nccRCC setting remains an unmet need. The field is evolving rapidly, with phase III trials of combination therapies targeting PD-1/PD-L1 and VEGF or CTLA-4, which could again change how we treat metastatic RCC.

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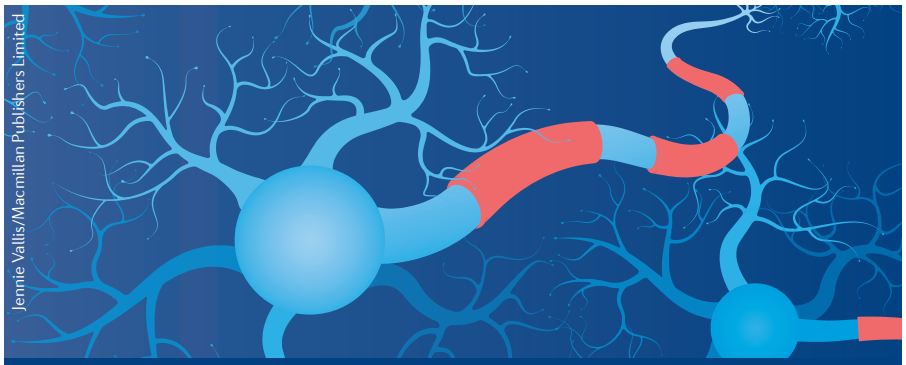

NEUROENDOCRINOLOGY IN 2016

Neuroendocrine control of metabolism and reproduction

Manuel Tena-Sempere

Neuroendocrine networks were previously perceived mainly as transcriptionally controlled, neural regulatory pathways that are centred at the hypothalamus. However, multisystemic circuits encompassing the brain and peripheral tissues have now been uncovered that involve nonneuronal cells and nontranscriptional regulatory mechanisms, with previously unidentified functions, such as reward and behaviour. Several developments in 2016 have helped to consolidate these new advances.

Interactions between the nervous system and hormones play an essential part in all facets of homeostasis and regulate key aspects of physiology. In turn, numerous diseases arise from perturbed neurohormonal pathways or their defective responses to different stressors. Notably, neuroendocrine control of energy, metabolic homeostasis and reproduction has drawn much attention, as prevalent disorders of escalating incidence, such as diabetes mellitus, obesity, pubertal alterations and infertility, have a substantial neuroendocrine dimension.

The classic view of neuroendocrinology emphasized a dominant role of neuronal circuits as targets and regulators of endocrine organs, with a major role for the hypothalamus as an integration centre and output pathway for the control of downstream endocrine systems. Similarly, transcriptional regulatory actions of hormones on neural pathways were stressed. Accordingly, neuroendocrine studies were dominated by protein or RNA expression analyses and invasive approaches, such as explant neurosecretion and electrophysiology, which often precluded implementation in truly physiological conditions.

Nevertheless, new conceptual and technical advancements have widened our understanding of neuroendocrine physiology, with considerable progress in the areas of metabolism and reproduction, including the discovery of essential regulators (such as the reproductive gatekeepers kisspeptins) and the application of novel functional techniques (such as optogenetics). These advances have enabled the identification of multisystemic regulatory pathways that affect the brain and peripheral tissues. These bidirectional neurohormonal interactions involve non-neuronal cells and nontranscriptional regulatory mechanisms, as well as previously unidentified functions (such as reward and behaviour) and areas (other than classic hypothalamic centres for feeding and reproductive control). Several studies published in 2016 will have a substantial influence in consolidating these new perspectives in neuroendocrinology.

Feeding drive is now recognized to derive largely from mechanisms that are linked to hedonic and reward responses, previously regarded as nonhomeostatic, but nonetheless also controlled by homeostatic signals. Starvation leads not only to compulsive feeding but also to high-risk behaviours characterized by aggression and fear. Padilla and co-workers[1] uncovered the neuronal pathway underlying some adaptive behaviours in the starved state, by showing that a subset of neurons in the hypothalamic arcuate nucleus (ARC), expressing agouti-related protein (AgRP), is also involved in the modulation of fear and aggression in conditions of starvation.

AgRP neurons are regulated by key metabolic hormones and transmit a potent orexigenic drive by projecting to neurons in other brain areas, such as the paraventricular nucleus (PVN). A subset of ARC AgRP neurons projecting to the medial amygdala (MeA) now also seems to be essential for promoting risk-taking behaviours and reducing territorialism in mice subjected to long-term food deprivation[1]. Although AgRP projections to PVN potently induce feeding, GABA-mediated inhibition of neurons in the MeA, driven by AgRP projections, is key for suppressing a downstream circuit reaching the posterior principle region of the bed nucleus of the stria terminalis, which causes reduced territorial aggressive behaviour and increased food-seeking activities. This switch from aggressive to nonaggressive behaviours is not caused by hunger itself but precisely controlled by the balance between activation and inactivation of the different subsets of AgRP neurons, illustrating the major role of these neurons as transducers of key metabolic hormones (such as leptin) to link feeding and behaviour[1].

Compelling evidence has suggested a role of non-neuronal cells, predominantly glia, in neuroendocrine regulation, and both metabolic status and leptin levels can influence the morphology and functionality of hypothalamic astrocytes[2]. García-Cáceres and co-workers[3] have now shown that insulin signalling in astrocytes plays a crucial part in brain glucose transport and sensing. Perturbations in

glycaemic control were observed following postnatal elimination of insulin receptors in astrocytes, demonstrating the importance of glial insulin signalling in systemic glucose metabolism. The data also support a major role for astrocytic insulin signalling in the regulation of ARC pro-opiomelanocortin (POMC) neurons, which are key in feeding and energy homeostasis, as the glial cell coverage, synaptic-input organization and function of POMC neurons were severely perturbed upon astrocytic loss of insulin receptors. This result primarily occurs via modulation of insulin and glucose uptake through the blood–brain barrier, which was severely affected by ablation of insulin receptors in astrocytes. Indeed, central glucose handling was not perturbed by insulin receptor elimination in glial cells, suggesting that perturbation of POMC neurons is mostly indirect. These findings change our understanding of the mechanisms of glucose sensing and insulin actions in the brain[3], and stress that conditions of gliosis, such as in obesity, might deregulate peripheral glycaemic control via alteration of astrocytic insulin signalling.

Besides transcriptional regulation, neuroendocrine functions, such as metabolism and reproduction, are also controlled by epigenetic mechanisms. Among these, non-coding RNAs, especially microRNAs (miRNAs), have received substantial attention. Previous data demonstrated a multifaceted role of miRNAs in metabolism and indirectly suggested their involvement in pubertal control[4]. This year, Messina and co-workers[5] showed that miRNA-dependent regulation of gonadotropin-releasing hormone (GnRH)-expressing neurons is key for changes in their neurosecretion that lead to puberty onset.

This team showed that elimination of Dicer, the enzyme that is responsible for generation of mature miRNAs, in GnRH neurons led to absence of puberty and severe central hypogonadism. Expression and functional analyses also revealed specific miRNA signatures in developing GnRH neurons, with prominent roles of miR-200 and miR-155 in the control of GnRH function[5]. Notably, rather than by direct inhibition of the *Gnrh* gene, these miRNA regulators operated mainly via repression of inhibitors of GnRH transcription, such as ZEB1 and CEBPB, which have other known neuroendocrine roles, including regulation of pituitary secretion of luteinizing hormone, or mediating the central neuroendocrine effects of nitric oxide[5]. These results foreshadow future analysis of deregulated miRNA signalling in perturbations of human puberty and other neuroendocrine axes.

In addition to central signals, peripheral hormones are essential in neuroendocrine control. Gut factors, such as ghrelin, peptide YY and glucacon-like peptide 1, are known regulators of metabolism, energy balance and reproduction. In 2016, Li and co-workers[6] demonstrated an important role for neurotensin, a 13-amino-acid neuropeptide produced in various tissues, including the brain and small intestine, in the control of body-weight homeostasis and fat deposition. Indeed, knockout of neurotensin protected mice from high-fat diet-induced obesity and prevented comorbidities of obesity, such as insulin resistance and hepatic steatosis.

In the brain, neurotensin is associated with hypothalamic leptin action and is regarded as an anorectic factor[7]. Neurotensin levels were increased in a rat model of body-weight loss by gastric bypass surgery, in which blockade of peripheral neurotensin increased feeding[7]. Hence, the protective phenotype of neurotensin knockout might seem to be counter-intuitive. According to Li *et al.*[6], neurotensin operates mainly in the periphery by modulating intestinal fat intake via an AMPK-mediated mechanism. Admittedly, other central neuroendocrine interactions of neurotensin cannot be ruled out. Yet, elevated circulating levels of pro-neurotensin are found in patients with obesity and insulin resistance, and doubled the risk of development of obesity later in life compared with nonobese individuals[6].

This year has also seen developments in technical approaches for sophisticated noninvasive exploration of neuronal activity, applicable to neuroendocrine functions. Stanley and colleagues[8] reported a novel method for remote, noninvasive, timed activation or suppression of hypothalamic circuits controlling glucose homeostasis and feeding, by the use of microwaves or magnetic fields in genetic models. The basis for this approach was reported in 2015 (REF. 9), but this study is the first to interrogate the physiological consequences of such neuronal manipulation on relevant neuroendocrine functions, such as feeding and metabolism. The technique enabled the modulation of peripheral glucose levels by manipulating glucosensing neurons in the ventromedial hypothalamus and complements other new techniques, such as optogenetics and designer receptors exclusively activated by designer drugs (DREADDs). The electromagnetic approach operates remotely, does not need implantable devices, reaches diffuse cell populations, and its cell activation kinetics might be superior to that of DREADDs. Hence, this new method provides opportunities for basic and translational research in neuroendocrinology, for both neuronal and endocrine cell types[8].

The breakthroughs highlighted here are illustrative of the large body of experimental and clinical neuroendocrinology research conducted in 2016. These studies foreshadow an exciting year of research in this field in 2017.

Key advances

- A key hypothalamic population of feeding-controlling AgRP neurons projects to areas of the amygdala to modulate fear and aggressive behaviours[1]
- Insulin signalling in hypothalamic astrocytic cells plays a key part in central glucose sensing and systemic control of glucose metabolism[3]
- A novel, nontranscriptional regulatory mechanism, involving a switch in the expression of specific miRNAs in GnRH neurons, is essential for proper timing of puberty onset[5]
- The gut–brain neuropeptide neurotensin plays a fundamental part in fat homeostasis, and ablation of neurotensin protects from excessive intestinal fat absorption and obesity in mice[6]
- A new system for noninvasive, timed activation or inhibition of neuronal pathways *in vivo*, which uses magnetic fields and microwaves, has been applied to hypothalamic glucosensing neurons [8]

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Competing interests statement
The author declares no competing interests.

ADIPOSE TISSUE IN 2016

Advances in the understanding of adipose tissue biology

Shingo Kajimura

Adipose tissues have a central role in energy homeostasis, as they secrete adipokines and regulate energy storage and dissipation. Novel adipokines from white, brown and beige adipocytes have been identified in 2016. Identifying the specific receptors for each adipokine is pivotal for developing greater insights into the fat-derived signalling pathways that regulate energy homeostasis.

White adipose tissue (WAT), which accounts for anywhere from 5% to 50% of human body weight, is a major source of endocrine signalling. As a testament to the importance of adipose tissue as a crucial endocrine organ, leptin therapy for individuals with generalized lipodystrophy who frequently develop severe hepatic steatosis, insulin resistance and diabetes mellitus has been approved by the FDA since 2014 (REF. 1).

Although generalized lipodystrophy is frequently associated with insulin resistance, patients with neonatal progeroid syndrome (NPS; Online Mendelian Inheritance in Man (OMIM) #264090) develop a rare form of partial lipodystrophy in which they remain insulin sensitive and euglycaemic. In 2016, Chopra and colleagues identified mutations in the gene that encodes profibrillin (*FBN1*) in patients with NPS; the mutations were found to disrupt the expression of asprosin, the C-terminal cleavage product of profibrillin[2]. Asprosin, which is a 140-amino-acid secreted polypeptide, is abundantly expressed in mature white adipocytes and is thus considered a new adipokine. The authors found that levels of asprosin were raised under fasting conditions in healthy humans and rodents. Remarkably, a single injection of recombinant asprosin to wild-type mice induced a significant increase in plasma levels of glucose and insulin within 30 min. Mechanistically, asprosin acts directly on the liver to stimulate hepatic glucose production by increasing intracellular cAMP levels, subsequently activating the protein kinase A (PKA) signalling pathway. Of note, plasma levels of asprosin (in the range of 5–10 nM) in humans with obesity and mouse models of obesity are higher than those in non-obese controls in concordance with insulin levels, which suggests that asprosin levels are associated with insulin resistance. Blocking asprosin action, via a neutralizing antibody against asprosin or genetic deletion of *Fbn1*, reduced plasma levels of insulin and hepatic glucose production *in vivo*. These data provide compelling evidence that asprosin is a fasting-induced adipokine that controls hepatic glucose production and insulin sensitivity. The study also suggests that there is an as yet uncharacterized receptor (or receptors) in hepatocytes through which asprosin acts to trigger cAMP–PKA signalling (FIG. 1). Blocking asprosin and its downstream pathways might be beneficial for the treatment of type 2 diabetes mellitus.

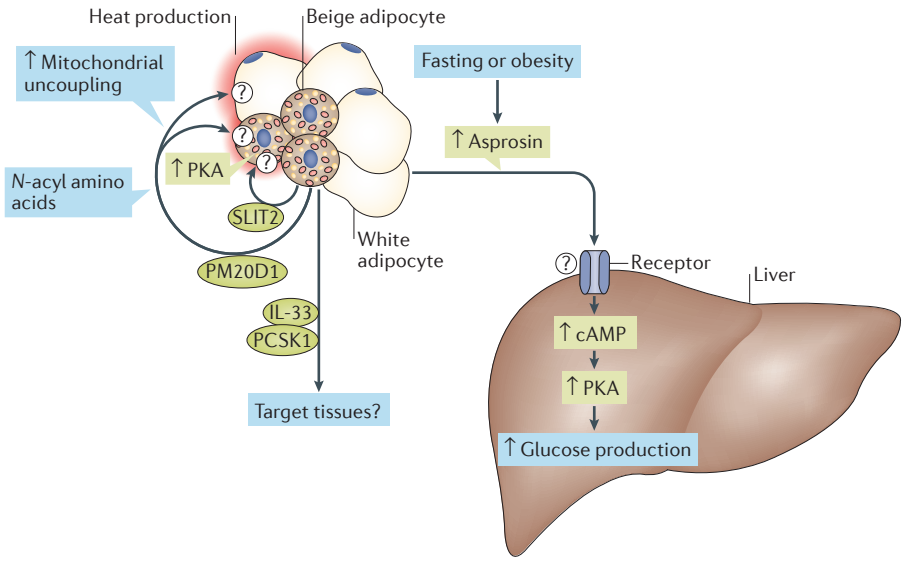

Figure 1 | **The regulation of systemic glucose homeostasis and insulin sensitivity through adipokines.** Newly identified secretory molecules from white, brown and beige adipocytes control thermogenesis, hepatic glucose production and/or insulin sensitivity. PCSK1, proprotein convertase subtilisin/kexin type 1; PKA, protein kinase A; PM20D1, peptidase M20 domain-containing protein 1; SLIT2, Slit homologue 2 protein.

In addition to WAT, mammals have brown adipose tissue (BAT), which dissipates energy in the form of heat and staves off hypothermia and obesity. Owing to the much smaller mass of BAT than WAT in the human body (~60 g of BAT in the average adult[3]), its contribution as an endocrine organ to whole-body homeostasis seems to be marginal. However, recent studies have reported on secretory molecules from BAT, so called 'batokines', which include fibroblast growth factor 21 (FGF21), neuregulin 4 (NRG4), vascular endothelial growth factor A (VEGFA) and bone morphogenetic protein 8B (BMP8B). These studies indicate a physiological role of BAT as an endocrine organ[4]. Notably, an 'inducible form' of thermogenic adipocytes, known as beige adipocytes, exists in adult humans[5]. Beige adipocyte differentiation can be induced by chronic cold exposure, exercise, cancer cachexia and bariatric surgery[6]. Given the recent animal studies showing its biological importance in the regulation of whole-body energy expenditure

Key advances

- A new adipokine, asprosin, promotes hepatic glucose production. Inhibition of pathologically raised levels of asprosin protects against obesity-linked hyperinsulinaemia[2]
- Transplantation of human beige adipocytes improves systemic glucose homeostasis in mice with diet-induced obesity[7]
- New batokines, PM20D1 and SLIT2, contribute to the regulation of whole-body thermogenesis and systemic glucose homeostasis[8,9]

and glucose homeostasis, it is probable that batokines mediate, at least in part, the metabolic improvements achieved by increased beige fat mass[6].

> These studies indicate a physiological role of BAT as an endocrine organ

In fact, studies published in 2016 support the above-described hypothesis. In a study by Corvera and colleagues, the authors obtained human beige adipocytes derived from the capillaries of subcutaneous WAT and then implanted the adipocytes into mice with diet-induced obesity[7]. The mice implanted with human beige adipocytes had lower levels of fasting blood glucose, increased glucose tolerance and reduced hepatic steatosis 7 weeks after the implantation compared with control mice implanted with matrigel (vehicle). Improved glucose tolerance following implantation of beige adipocytes was associated with an increased glucose turnover rate and adiponectin secretion from the transplanted human beige adipocytes. Notably, the authors identified several secretory factors, such as proprotein convertase subtilisin/kexin type 1 (encoded by *PCSK1*), its substrate proenkephalin (encoded by *PENK*) and IL-33 (encoded by *IL33*), which were abundantly expressed in the human beige adipocytes. This study reinforces the notion that increased beige fat mass affects whole-body glucose homeostasis; however, determining the causal link between these secretory molecules and the metabolic improvement that is achieved by implanting beige adipocytes requires further investigation (FIG. 1).

Two further studies published in 2016 have identified previously unknown batokines, peptidase M20 domain-containing protein 1 (PM20D1) (REF. 8) and Slit homologue 2 protein (SLIT2) (REF. 9), which regulate whole-body energy expenditure and glucose homeostasis (FIG. 1). Long and co-workers used global transcriptional profiling and identified PM20D1 as a secretory enzyme that was highly enriched in mitochondrial brown fat uncoupling protein 1 (UCP1)-positive cells (that is, brown and beige adipocytes)[8]. When overexpressed in mice, PM20D1 catalyses the synthesis of *N*-acyl amino acids from free fatty acids, thereby increasing whole-body energy expenditure, which leads to reduced fat mass and body weight. The authors further showed that *N*-acyl amino acids increase cellular respiration and reduce body weight by functioning as a mitochondrial uncoupler. As the thermogenic effect was found in UCP1-positive and UCP1-negative cells, *N*-acyl amino acids probably act not only on brown and beige adipocytes but also on neighbouring cells (FIG. 1). Notably, the authors identified several mitochondrial proteins, including ANT1 and ANT2, that bind directly to *N*-acyl amino acids. Further studies are needed to determine the mechanisms by which *N*-acyl amino acids are taken up into the target cells and stimulate mitochondrial uncoupling.

Svensson *et al.* used quantitative proteomics to search for molecules that are secreted by beige adipocytes[9]. The authors found that the C-terminal fragment of SLIT2 (SLIT2-C) is cleaved from the full-length SLIT2 protein; SLIT2-C activates thermogenesis in BAT and beige adipose tissue. It does so by activating the PKA signalling pathway, which is a well-known downstream pathway, in response to cold exposure and β-adrenergic receptor activation. Although these molecules are enriched in brown and beige adipocytes, they are also expressed in non-adipose tissues. Thus, further analyses using tissue-specific knockout mouse models are needed to determine the relative contributions of the secretory factors derived from BAT and beige adipose tissue versus alternative tissues in the regulation of whole-body metabolism.

As we learn more about the various roles of beige adipocytes in thermogenesis, glucose metabolism and lipid homeostasis, it might be conceivable that multiple types of beige adipocytes with distinct functional roles exist within adipose tissue. In this regard, a paper by Graff and colleagues used a variety of Cre-inducible mouse lines to critically investigate the developmental origins of beige adipocytes in response to cold exposure[10]. The authors found that perivascular mouse cells expressing smooth muscle actin gave rise to 60–68% of UCP1-positive beige adipocytes, whereas existing mature white adipocytes did not give rise to beige adipocytes. These data might indicate that additional lineages of beige adipocytes exist within a WAT depot.

> …identification of their specific receptors will be an important future theme in the field

In summary, the new studies published in 2016 made important advances in the field of adipose biology with the identification of new signalling entities from adipocytes that control systemic energy balance. Clearly, the identification of their specific receptors will be an important future theme in the field; this will enable us to determine their target tissues and/or cells and the underlying mechanisms of their actions.

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The author declares no competing interests.

GENETICS OF T2DM IN 2016

Biological and translational insights from T2DM genetics

Mark I. McCarthy

Type 2 diabetes mellitus (T2DM) is a major global health challenge. Development of more effective strategies for prevention and therapy depends on an improved understanding of its pathogenetic mechanisms. 2016 ends a period during which large-scale discovery of risk alleles for T2DM became routine and heralds a shift in research focus towards their exploitation to fuel mechanistic insights.

Over the past decade, the dominant approach to genetic discovery for complex traits has involved genome-wide surveys of (mostly) common genetic variants using array-based genome-wide association studies (GWAS). Successive GWAS have identified >100 loci that are robustly associated with type 2 diabetes mellitus (T2DM). The risk variants at these loci typically have modest effects on T2DM predisposition and collectively account for only 10% of overall disease risk, far short of the proportion that twin and family studies indicate is attributable to overall genetic variation[1].

A range of explanations have been proffered for this disparity (often described as 'missing heritability'). One of these explanations implicates risk alleles of low frequency — these alleles are mostly invisible in existing GWAS, as these have primarily interrogated common variants, but can be identified through the more comprehensive variant evaluation that is provided by next-generation sequencing.

Fuchsberger and colleagues[2] describe the first large-scale use of sequencing to explore risk of T2DM, combining whole-genome sequencing data from 2,657 controls and T2DM cases with whole-exome sequencing data from a further 12,940 individuals. These data were extended, capturing a subset of the sequence-detected variants, by genotyping 111,548 additional individuals. This study identified many statistically significant association signals for T2DM; however, almost all were common, and many lay within existing GWAS signals. Where the risk variants mapped to protein-coding sequence, these discoveries provided valuable clues regarding which regional transcript was driving GWAS signals hitherto detected through non-coding (regulatory) variation.

Evidence supporting a contribution of lower frequency variants to the risk of T2DM was scant. The only compelling signal involved an excess of rare loss-of-function alleles within the set of genes that were previously implicated in monogenic diabetes mellitus. The paucity of low frequency association signals suggests little or no selective pressure for T2DM risk alleles during human prehistory. Evidence that most variation in individual risk of T2DM is found in common variants limits the potential to reassign individuals with T2DM into discrete disease subtypes.

Although rare variants are unlikely to explain much of the risk variance, their identification and characterization remain important objectives. Rare alleles detectable in genome-wide studies will, for reasons of statistical power, generally have large phenotypic impact, and such discoveries often lead to clear and rapid mechanistic insights. Whereas few examples of high-impact common variants in outbred populations exist, such variants can rise to higher frequency in isolated populations, through chance or local adaptation[3].

An example of a high-impact, geographically restricted variant emerged in 2016. Minster and colleagues conducted a GWAS for BMI in people from Samoa, a population at high risk of obesity and T2DM[4]. The key finding was a replicated association signal localized to a coding variant in *CREBRF*, a gene implicated in fat deposition. The BMI-raising allele has a frequency of 27% in people from Samoa but is essentially absent outside the Pacific islands. This frequency difference might reflect selection for a 'thrifty' allele that, by promoting efficient fat storage, enhanced the survival prospects of carriers during the perilous transoceanic migrations that led to the peopling of the Pacific islands.

Intriguingly, the BMI-raising allele in *CREBRF* was associated with reduced, rather than increased, risk of T2DM. Such variants, which 'row against the epidemiological current', can provide instructive insights into the mechanistic relationships between otherwise highly correlated phenotypes. The probable explanation here is that risk of T2DM reflects not so much total adiposity but the distribution of that fat. Efficient storage of excess calories in safe adipose sites, promoted by the *CREBRF* allele, lowers the risk of T2DM because it reduces the opportunities for fat deposition in ectopic sites, such as the liver, that are more metabolically harmful. A recent study of insulin-resistance associated alleles in European populations powerfully supports this inference[5].

The major motivation behind genetic discovery for complex traits is the value of risk variants to reveal mechanisms that are central to disease pathogenesis (FIG. 1). Securing these mechanistic inferences has, until very recently, been a major challenge for T2DM. As with other common diseases, most GWAS signals map to regulatory sequences with few clues to the effector genes through which those variants act. In T2DM, many GWAS loci have predominant effects on insulin secretion, focusing attention on the pancreatic islets; because of their relative inaccessibility, the epigenomic and transcriptomic characteristics of pancreatic islets have been poorly captured by projects such as ENCODE and GTEx.

However, the use of sequence-based omics technologies to generate regulatory and expression maps for the human islet has demonstrated that T2DM risk variants are preferentially located within active islet enhancers. One T2DM GWAS region where this information has generated valuable insights lies next to the gene *MTNR1B*, which encodes one of the receptors for melatonin. The probable causal variant disrupts a NEUROD1 binding site in an islet enhancer

Key advances

- Disease-associated genetic variation can provide crucial insights into disease pathogenesis[2,4]
- Most of the variation in inherited predisposition to common diseases such as type 2 diabetes mellitus depends on common, shared variants, which mostly have modest effects; however, high-impact variants can be found in isolated populations[2,4]
- The intersection of genetic and genomic information from relevant tissues is driving the characterization of pathogenetic mechanisms[2,4,7]
- Genetic information is increasingly informing translational efforts, including the capacity to evaluate the contribution of external processes to disease prevalence[9]

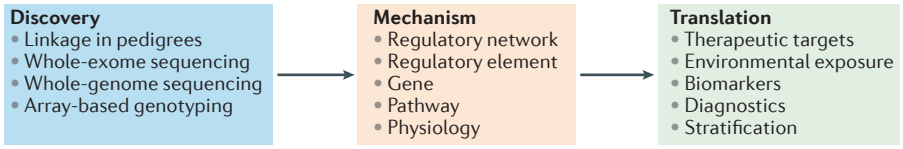

Figure 1 | **The identification of DNA sequence variants associated with type 2 diabetes mellitus.** A combination of genotyping and sequencing drives mechanistic insights that can reveal the molecular and physiological basis of disease. In turn, those insights provide a foundation for a range of translational opportunities that might support novel preventive and therapeutic strategies.

upstream of the gene, and variation at this site influences *MTNR1B* expression[6], highlighting some of the key machinery that connects the GWAS-detected variant to its influence on T2DM predisposition.

Tuomi and colleagues[7] put further flesh on the bones of this story, demonstrating that melatonin inhibits cAMP generation in islets and blocks insulin release. In a genotype-based recall study, this group showed that the capacity of melatonin to reduce insulin secretion was enhanced in homozygous carriers of the T2DM risk allele. The implication, that excessive melatonin action is detrimental to islet function, seems to be consistent with the idea that nocturnal secretion of melatonin has the benefi cial physiological effect of dialling down insulin production at night, when food intake is reduced.

However, the story is undoubtedly more complex. Loss-of-function coding variants in *MTRN1B* have been associated with increased, rather than reduced, risk of T2DM, and some epidemiological studies suggest a similar directional relationship[8]. These contrasting views probably reflect differences between the impact of an islet-specific GWAS variant and coding variants with wider tissue effects. Thus, the *MTNR1B* example not only illustrates the growing range of data that can support the assignment of function to T2DM-associated genetic variants but also highlights the biological complexity that must be addressed.

Mechanistic inference provides a crucial first step towards translation, defining novel therapeutic targets and highlighting potential biomarkers. However, genetics offers additional clinical opportunities. Genetics can help to design more effective preventive strategies, for example, through the use of Mendelian randomization approaches to identify the specific components of the Western lifestyle that have the strongest influence on risk of T2DM. In this regard, interest is growing in the role of the gut microbiome (and of dietary composition) in promoting obesity and T2DM.

The picture remains confused. Associations between obesity and T2DM and the gut microbiome content and diversity are easy to detect, but whether such differences are causal or reactive is far from clear. The work of Pedersen and colleagues[9] provides an important contribution to this story, connecting insulin resistance, raised levels of circulating branched-chain amino acids (BCAAs) and a microbiome content that promotes BCAA synthesis. With parallel efforts to identify genetic variants that influence microbiome content and diversity[10], it is now becoming possible to establish the mechanistic relationships between microbiome content, BCAA levels and the risk of T2DM.

The field has advanced beyond recognition in the decade since GWAS arrived. Much remains to be discovered, but the combination of comprehensive elucidation of risk variants, harnessed to genomic information from relevant tissues and physiological studies in humans and animal models, sets the scene for important translational advances in the coming years.

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EXERCISE METABOLISM IN 2016

Health benefits of exercise — more than meets the eye!

Mark A. Febbraio

Although regular physical activity can prevent or reduce the risk of many age-related diseases, the molecular mechanisms underpinning the protective effects of exercise are largely unknown. In 2016, a series of studies demonstrated that crosstalk between tissues during exercise can protect against metabolic disease, cancer, retinal degeneration and memory loss. These studies provide a molecular basis for the concept of 'exercise as medicine'.

Since ~450 BC, physical activity has been known to be able to prevent chronic disease. Quotes attributed to Hippocrates, the father of Western medicine, include: "Walking is man's best medicine" and "If there is a deficiency in food and exercise the body will fall sick". Now, even short periods of physical inactivity are known to be associated with disruption of metabolic homeostasis, which manifests as decreased insulin sensitivity, reduced

postprandial lipid clearance, loss of muscle mass and accumulation of visceral adiposity[1]. These acute changes provide a link between physical inactivity and an increased risk of developing many diseases, including type 2 diabetes mellitus (T2DM), cardiovascular disease, cancers such as those of the colon and breast, osteoporosis, osteoarthritis, erectile dysfunction and polycystic ovary syndrome[1,2]. The benefits of physical activity have been attributed to several mechanisms, which include reduced adiposity, increased cardiorespiratory fitness (maximal oxygen consumption (VO_2 max)), reduced levels of circulating lipids and the maintenance of muscle mass. However, in the current millennium, research has shown that, during exercise, proteins, peptides, enzymes and metabolites are released from one organ (mainly contracting skeletal muscle) to affect the metabolism in another organ.

In 2016, this paradigm was strengthened in several important studies. As exercise involves muscle contraction, most emphasis has been placed on the release of proteins from contracting skeletal muscle (so called myokines) that affect metabolic processes in other organs[3]. Exercise is known to improve brain function and cognition. In an elegant and important study, Moon *et al.*[4] initially treated L6 myotubes with the AMP-activated protein kinase (AMPK) agonist 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) in an attempt to model the effects of exercise *in vitro*. Subsequent screening of the culture media for proteins using mass spectrometry revealed the presence of cathepsin B. The investigators validated cathepsin B as a myokine by demonstrating that the levels of this protein increased in the plasma of mice, monkeys and humans during exercise. In humans, plasma levels of cathepsin B correlated with both fitness and memory. Moreover, although running improved memory and increased hippocampal neurogenesis in wild-type mice, no effect was observed in mice deficient in cathepsin B[4]. This study demonstrated that exercise can induce the release of cathepsin B from contacting skeletal muscle to modify memory and brain function (FIG. 1), which validates the hypothesis that exercise is beneficial for delaying dementia in ageing.

Although contracting muscle is undoubtedly an organ capable of releasing important proteins and metabolites during exercise, other organs could have endocrine-like properties during physical activity. In a complex study published in 2016, Mera *et al.*[5] demonstrated that bone can also drive the adaptation to physical exercise via the release of osteocalcin. The investigators demonstrated

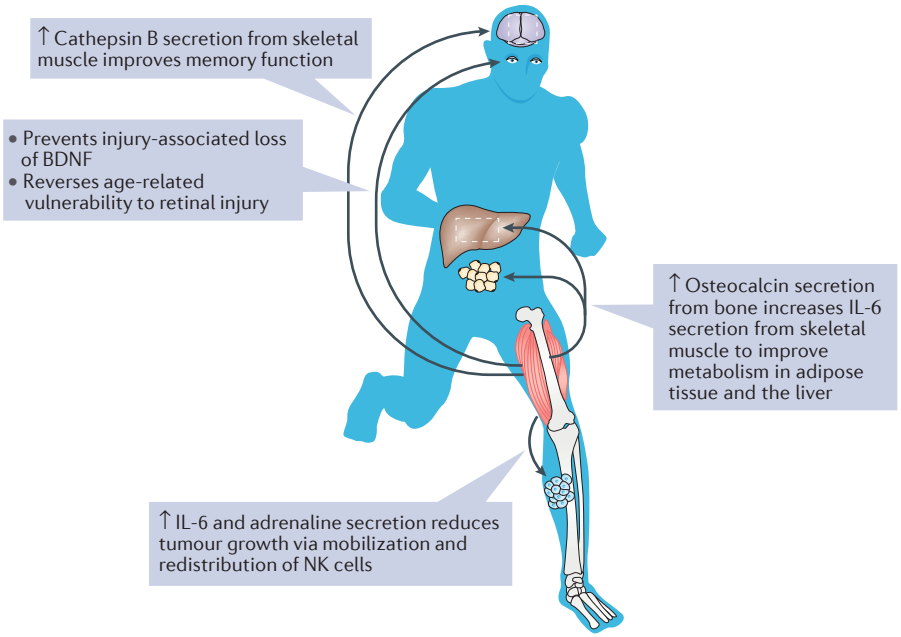

Figure 1 | **The multiple benefits of exercise.** Exercise can reverse age-related vulnerability to retinal injury, increase memory function via the myokine cathepsin B, improve metabolic homeostasis via communication between bone and skeletal muscle, and reduce tumour growth via the mobilization and redistribution of natural killer (NK) cells. BDNF, brain-derived neurotrophic factor.

that circulating levels of undercarboxylated and bioactive osteocalcin markedly increase during exercise. Circulating osteocalcin was then shown not only to increase intramuscular glucose uptake during exercise but also to increase the production and release of the prototypical myokine IL-6, in turn increasing fatty acid availability from adipocytes and glucose production in the liver; findings that support previous studies performed over a decade ago[6,7]. The study by Mera *et al.*[5] is an important addition to the existing model of tissue crosstalk, as it uncovered a bone–muscle–liver and/or adipose tissue axis that regulates nutrient supply and demand during muscle contraction (FIG. 1). Determining precisely how bone senses muscle contraction is the next challenge in further defining this model.

Physical activity can decrease the risk and/or improve the prognosis of a limited number of cancers such as those of the colon, breast and possibly endometrium[2]. Many hypotheses have been proposed as to the mechanism underpinning the beneficial effects of physical activity on carcinogenesis. These hypotheses include: the mechanistic target of rapamycin (mTOR) network hypothesis, in which exercise inhibits carcinogenesis by suppressing activation of mTOR signalling in mammary carcinomas; the hormesis hypothesis, in which the carcinogenic response to physical activity is nonlinear and is accounted for by a physiological cellular stress response; and the metabolic reprogramming hypothesis, in which exercise limits the amount of glucose and glutamine available to mammary carcinomas, thereby inducing apoptosis due to reversal of tumour-associated metabolic programming[8]. In an important paper published in 2016, Pedersen *et al.*[9] demonstrated that exercise initiates complex hormonal and immunological responses that inhibit tumour growth in mouse models of cancer. Tumour-bearing mice were randomly assigned to cages with access to either locked or unlocked running wheels. In the latter group, the mice ran considerably longer distances over a 4-week period than mice in the sedentary (locked wheel) group. Importantly, exercise-trained mice exhibited a >60% reduction in tumour incidence and growth across five different cancer models[9]. The investigators demonstrated that, by activating the sympathetic nervous system and increasing the exercise-induced IL-6 response, a specific subset of natural killer (NK) cells were immobilized. These NK cells were subsequently redistributed to the site of tumours to control tumour growth[9] (FIG. 1). This study is important for many reasons. First, if patients with cancer can tolerate physical exercise, clinical oncologists could integrate physical activity into current treatment plans, which could have profound effects on current lifestyle intervention for successful cancer treatment. Second, as with the studies on memory and metabolic

Key advances

- Cathepsin B is a contraction-induced myokine that improves memory function[4]
- Osteocalcin is released from bone during exercise to signal to skeletal muscle to release IL-6, which in turn regulates metabolic homeostasis[5]
- Exercise can reduce tumour growth in a variety of cancers in mice by mobilizing and redistributing natural killer cells[9]
- Exercise prevents the loss of brain-derived neurotrophic factor in the retina after injury to preserve neuronal function[10]

homeostasis described earlier[4,5], the study by Pedersen and colleagues provides a molecular basis for the concept of 'exercise as medicine'. Such evidence-based research might have profound public health ramifications that facilitate behavioural modification within patient populations.

Last, in an intriguing study, Chrysostomou *et al.*[10] demonstrated that exercise can reverse age-related vulnerability to retinal damage. Retinal ganglion cells are recognized to be increasingly susceptible to injury with advanced age. As levels of brain-derived neurotrophic factor (BDNF) and AMPK both increase with exercise and are thought to mediate the beneficial effects of exercise, the investigators focused on the role of these two molecules in the protective effects of exercise against retinal injury in mice[10]. The investigators demonstrated that retinal ganglion cells undergo an increase in intra-ocular pressure during exercise, which in turn preserves inner retinal synapses. Moreover, when an injury takes place in sedentary mice, levels of BDNF normally decrease, but this reduction did not occur in exercised mice. However, in mice with *BDNF* haploinsufficiency or when BDNF was pharmacologically blocked, the beneficial effect of exercise was diminished[10]. Interestingly, although BDNF can activate AMPK in skeletal muscle during exercise, the protective effects of BDNF in this model were independent of activation of this important fuel-sensing kinase[10]. Chrysostomou and colleagues justifiably concluded that their data provided new insights into the mechanism underlying exercise-mediated protection of retinal cells (FIG. 1).

In summary, these four important studies published in 2016 provide new insights into the molecular mechanisms underlying the protective effects of exercise against a myriad of diseases including dementia[4], cancer[9], obesity[5] and retinal disease[10]. In addition, by uncovering these mechanisms, the investigators have opened up the field for future identification of therapeutic targets and development of therapies for the treatment of these diseases. However, more importantly, these studies provide additional evidence that 'exercise is medicine'.

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The author declares no competing interests.

FGF21 AND METABOLIC DISEASE IN 2016

A new frontier in FGF21 biology

Matthew J. Potthoff

In 2016, four studies were published that provided crucial new information on the endocrine actions of the hormone fibroblast growth factor 21 (FGF21). These studies provide a framework for the nutritional stimuli that regulate *FGF21* expression and demonstrate a major role for FGF21 in primates and humans in regulating food intake, macronutrient preference and central reward pathways.

Fibroblast growth factor 21 (FGF21) is an endocrine hormone that regulates energy homeostasis and insulin sensitivity[1]. FGF21 is produced by the liver in response to various nutritional, physiological and pathological stimuli, and signals to multiple tissues including the central nervous system (CNS) and adipose tissues to mediate metabolic effects on carbohydrate and lipid metabolism[1]. Extended pharmacological administration of FGF21 to obese or diabetic rodents increases energy expenditure and browning of adipose tissues, and markedly reduces body weight without significantly affecting food intake[1]. The identification of FGF21, and the subsequent characterization of its function, is a remarkable story that has been full of unexpected twists and turns. Although FGF21 was identified in a screen for factors that induce glucose uptake in white adipocytes *in vitro* through insulin-independent mechanisms, FGF21 was subsequently found to profoundly enhance insulin sensitivity *in vivo*. Notably, despite being identified as a factor that regulates glucose metabolism, the most striking and consistent metabolic effects of FGF21 across species are on lipid metabolism in animal models of obesity and diabetes mellitus[1]. In 2016, a new chapter in the FGF21 story unfolded, as four papers were published that provide novel insights into the production and function of FGF21 in mice, monkeys and humans.

Talukdar and colleagues evaluated the metabolic effects of a long-acting FGF21 analogue, PF-05231023, in monkeys with obesity and humans with obesity and type 2 diabetes mellitus[2]. Consistent with a previous study examining the effects of a different FGF21 analogue in humans[3], Talukdar *et al.* observed marked decreases in body weight in both humans and monkeys with obesity in response to PF-05231023 administration[2]. Surprisingly, in contrast to the metabolic effects of this FGF21 analogue in rodents (that is, increased weight loss without decreased food intake), PF-05231023 reduced body weight in monkeys by decreasing food intake without affecting browning of subcutaneous white adipose tissue. Administration of PF-05231023 to monkeys resulted in a marked decrease in food intake; pair feeding untreated obese monkeys with the same calorie content per day as the 10 mg/kg PF-05231023 treatment group resulted in body weight changes of the same magnitude as obese monkeys administered PF-05231023. Similar metabolic effects were observed in humans following PF-05231023 administration, which included an ~4–5% decrease in body weight after only 25 days[2]; food intake data were not reported. Thus, the effect of FGF21 on body weight that was identified in rodents seems to translate to primates and humans. However, the mechanism underlying this effect might be species-specific, with energy expenditure being the primary mechanism to reduce body weight in rodents and food intake the primary mechanism in primates and humans (FIG. 1).

Although administration or overexpression of FGF21 in rodents does not decrease total caloric intake, von Holstein-Rathlou and colleagues demonstrated that FGF21 regulates macronutrient intake in mice[4]. Previous work in humans identified single-nucleotide polymorphisms (SNPs) near the *FGF21* locus that are associated with changes in macronutrient intake, including increased carbohydrate intake and decreased fat intake[5,6]. Using both gain-of-function and loss-of-function animal models, von Holstein-Rathlou *et al.* discovered that FGF21 regulates simple sugar intake and preference but not the intake of lipids or protein[4]. Mice lacking FGF21 exhibited increased simple sugar intake, whereas mice overexpressing FGF21 had markedly reduced sugar consumption, without decreased total caloric intake. Acute administration of recombinant FGF21 protein to wild-type mice rapidly and significantly suppressed simple sugar intake. Interestingly, exogenous FGF21 administration also suppressed the intake of the non-caloric sweetener sucralose. von Holstein-Rathlou and colleagues found that FGF21 production by the liver is increased by high carbohydrate levels through activation of the transcription factor ChREBP. FGF21 then enters the circulation and signals to paraventricular neurons in the hypothalamus to reduce sugar intake. These effects of FGF21 were not mediated by taste sensing but, rather, through taste processing[4]. Together, these data reveal that FGF21 functions as a key mediator of a novel liver-to-brain hormonal axis that regulates macronutrient preference by acting as a sugar satiety signal (FIG. 1).

Key advances

- Administration of an FGF21 analogue to primates markedly decreases body weight and food intake but does not increase browning of adipose tissues[2]
- FGF21 mRNA and protein expression is induced in the liver, and the protein enters the circulation in response to low levels of protein and high levels of carbohydrate[4,9]
- Elevated levels of FGF21 signal to the brain to suppress sugar intake and sweet taste preference[4,7]
- FGF21 decreases central reward and reduces alcohol preference[7]

Consistent with the role of FGF21 in regulating sugar intake, a second study by Talukdar and colleagues also found that FGF21 suppresses sweet taste preference by signalling to the CNS[7]. However, in contrast to the study of von Holstein-Rathlou and colleagues, Talukdar *et al.* extended their studies to monkeys and found that administration of the long-acting FGF21 analogue, PF-05231023, potently inhibited sweet taste preference[7]. Interestingly, Talukdar *et al.* also discovered that overexpression of FGF21 in mice reduced alcohol preference, which suggests that FGF21 affects central reward. Indeed, long-term administration of FGF21 in mice decreased levels of dopamine in the nucleus accumbens and the ventral tegmental area, key brain regions that regulate central reward[7]. Importantly, this role of central FGF21 signalling in the regulation of alcohol consumption seems to translate to humans, as a SNP in *KLB* (encoding the FGF21 obligate co-receptor β-klotho) is associated with alcohol consumption[8]. Increased levels of this liver-derived hormone might thus be produced in response to excess levels of carbohydrate and/or alcohol to suppress reward and prevent alcohol-induced and non-alcohol-induced liver injury (FIG. 1).

Although FGF21 gene and protein expression is induced by high levels of carbohydrate to regulate nutrient metabolism[4], FGF21 is also induced during a number of different physiological conditions (including fasting and overfeeding) and in response to other diets (such as ketogenic diets, low-protein diets and high-fat diets)[1,9]. Explaining this paradoxical elevation of FGF21 levels has been difficult. To identify the nutritional and metabolic context for FGF21 induction, Solon-Biet and colleagues used the geometric framework, a multidimensional framework of 25

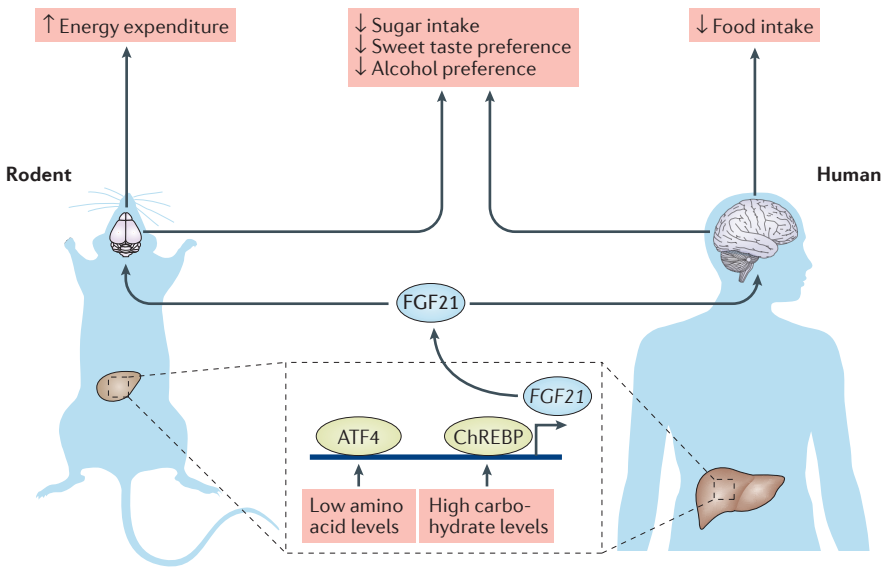

Figure 1 | **Liver-to-brain hormonal axis regulating energy homeostasis.** Production of circulating levels of FGF21 from the liver are induced in response to low protein or high carbohydrate levels in both rodents and humans. The conserved and species-specific effects of FGF21 signalling to the brain are indicated. ATF4, activating transcription factor 4; ChREBP, carbohydrate-responsive element-binding protein; FGF21, fibroblast growth factor 21.

> …FGF21 functions as a key mediator of a novel liver-to-brain hormonal axis…

diets varying in macronutrient (protein, carbohydrate and fat) and total-energy density, to assess the contribution of macronutrient intake and energy intake to hepatic and circulating levels of FGF21 in mice[9]. This comprehensive analysis revealed that maximal FGF21 induction was associated with a combination of low protein and high carbohydrate intake. Interestingly, FGF21 expression was not significantly altered by fat intake or total energy intake. Consistent with the *in vivo* data, incubation of human HepG2 liver cells with glucose increased FGF21 protein expression, and maximal levels were observed under conditions of high glucose levels and low branched-chain amino acid levels[9] (FIG. 1). These data are thus consistent with the observation that low protein[10] and high carbohydrate[4] levels induce FGF21 expression in humans and align with the model of von Holstein-Rathlou *et al.*, which proposed that FGF21 functions as a negative-feedback satiety signal to maintain macronutrient balance through suppression of simple sugar intake[4].

In summary, four important studies published in 2016 have shifted our understanding of the major physiological mechanisms controlling FGF21 expression and the physiological and pharmacological consequences of FGF21 signalling to the CNS. Additional studies are necessary to determine how the effects of FGF21 on macronutrient intake are retained across species despite species-specific differences in total caloric intake and energy expenditure (FIG. 1). Future studies elucidating the central pathways mediating FGF21 functions might yield important therapeutic targets to treat metabolic disease and drug addiction.

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Competing interests statement
The author declares no competing interests.

HEALTHY AGEING IN 2016

Obesity in geroscience — is cellular senescence the culprit?

Claudio Franceschi

Obesity and ageing are major worldwide health challenges associated with lifestyle changes and an increase in age-related diseases, characterized by chronic inflammation dubbed metaflammation and inflammaging. However, the mechanistic link between these inflammatory processes is still unknown. New findings in 2016 shed light on these issues and indicate common targets for intervention.

The obesity epidemic and the increase in the number of elderly people are currently presenting major worldwide health challenges. These phenomena have been attributed to rapid changes in lifestyle in the past century, which have created favourable conditions for survival but also access to nutrient-rich food and a sedentary lifestyle. As obesity is a powerful risk factor for many age-related diseases, a clash between these two trends can be predicted, which questions the optimistic vision of an endless increase of life expectancy and health span. Understanding the determinants of healthy ageing is crucial to avoid health-care systems becoming overwhelmed with sick elderly individuals. This concern has prompted a reappraisal of geriatrics or gerontology, known collectively as 'geroscience' (REF. 1). As ageing is the single major risk factor for age-related diseases, a central tenet of geroscience suggests that a small number of mechanisms (such as, cell senescence and inflammation[2,3]) are shared between these diseases and advancing age. This comprehensive approach offers a rationale to combat age-related diseases under a single umbrella of ageing, instead of individually, which is currently favoured by medical schools[1].

Several papers published in 2016 have enhanced our understanding of ageing and obesity, offering a strong, integrated rationale for their prevention and cure, as suggested by 'rejuvenation' seen in experiments of heterochronic parabiosis[2,3]. The well-known, but oversimplified, notion is that nutrient-rich, high-fat diets (HFDs) induce complex mechanical, vascular and/or hypoxic and endoplasmic reticulum stress in visceral adipocytes leading to the accumulation of inflammatory cells. These cells are thought to be responsible, at least in part, for the chronic inflammation in metabolic diseases called 'metaflammation' (REF. 4) and to contribute to a whole-body pro-inflammatory state that occurs during ageing, termed 'inflammaging' (REFS 2,3). These processes lead to cardiometabolic, degenerative and inflammatory disorders during ageing (FIG. 1). However, the mechanisms linking stressed adipose tissue and immune activation are still largely unclear.

In one study, a diet enriched with saturated fat, cholesterol and high-fructose corn syrup (roughly equivalent to a human fast-food diet) induced deleterious changes in body weight and composition, as well as in measures of physical, cardiac and metabolic health in mice, which were attributed to changes in cellular senescence[5]. This diet induced a dramatic increase in the number of cells expressing markers of cellular senescence (such as, p16, p53 and senescence-associated β-galactosidase (SAβ-gal)) in visceral adipose tissue (VAT) (FIG. 1). Senescent cells secrete cytokines, chemokines, matrix remodelling proteases and growth factors, which are collectively known as a senescence-associated secretory phenotype (SASP)[2]. A 7.5-fold increase in the number of VAT cells expressing CD68, a pan-macrophage marker, was observed, suggesting that these senescent cells were indeed macrophages. Importantly, physical exercise prevented accumulation of senescent cells and development of SASP, while concomitantly neutralizing the harmful effects of this diet on metabolic (weight, subcutaneous fat and VAT, insulin and glucose levels) and health (cardiac function and running capacity) parameters.

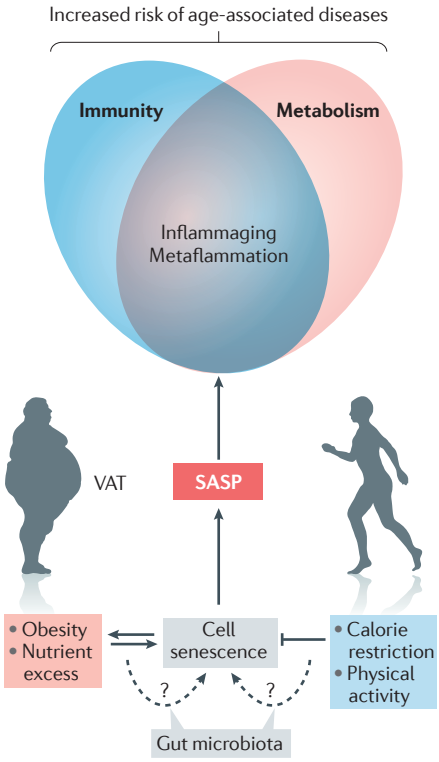

Figure 1 | **A nutrient-rich, high-fat diet induces accumulation in visceral adipose tissue of senescent immune cells (macrophages and T cells).** Senescent immune cells in visceral adipose tissue (VAT) are characterized by the secretion of a broad repertoire of active molecules impinging on several targets (cell growth, tissue repair, inflammation), referred to as the senescence-associated secretory phenotype (SASP). The pro-inflammatory activity of SASP contributes to metabolic inflammation (known as metaflammation) and to age-associated, low-grade, chronic inflammation (known as inflammaging), that in turn favour the onset of chronic age-related diseases. These factors might also be modulated by the gut microbiota.

Another article showed that, in addition to an increased number of macrophages (F4/80$^+$), senescent T cells preferentially accumulate in the inflammatory foci of VAT in HFD-fed mice[6]. These T cells constitutively expressed CD153$^+$PD1$^+$CD44^{high}CD62^{low}CD4$^+$, and ~60% expressed SAβ-gal. Interestingly, upon cell transfer, these senescent T cells induced inflammation in VAT (high expression levels of IL6 and TNF) and insulin resistance to lean mice, suggesting that these cells are likely to have a causal role in the development of obesity.

A third paper analysed the clearance of human senescent fibroblasts implanted into severe combined immunodeficient (SCID) mice, which were embedded into alginate beads to protect them from immunocyte attack[7]. One of the major cell types attracted by these senescent fibroblasts was a subpopulation of macrophages (F4/80$^+$) expressing p16 and SAβ-gal, which resembled senescent cells. These cells were sensitive to liposomal clodronate, which was used to selectively kill cells capable of phagocytosis. Moreover, systemic clodronate treatment of old animals reduced the number of cells expressing p16 and SAβ-gal, indicating that a significant proportion of these cells were indeed a subclass of macrophages.

Yang *et al.* showed that a moderate calorie restriction was effective in non-obese adult humans (aged 20–50 years)[8]. After 2 years of 25% calorie restriction, individuals had a 10.4% reduction in weight, an increase in autophagy markers in muscle and a significant reduction in circulating inflammatory markers (C-reactive protein: −40%; TNF: −50%), without any effect on immune responses (such as, skin delayed hypersensitivity and vaccine responsiveness).

Finally, Collins *et al.* investigated the relationship between compromised muscle integrity, diet, obesity-associated chronic inflammation, adipose tissue and the gut microbiota following a short-term, high-fat, high-sucrose diet[9]. In male Sprague Dawley rats, intramuscular fat, fibrosis and the number of pro-inflammatory cells (quantified by Oil Red O, picrosirius red staining and CD68$^+$ cells) increased (from ~0% to 1–2%, from ~2% to 4% and from ~1% to 6%, respectively) after 3 days and was sustained over 28 days. These findings were associated with systemic inflammatory changes and consistent, dynamic, alterations in gut microbiota composition.

These data indicate a number of key points. First, senescent macrophages have a major role in both ageing and obesity, and are potential cellular targets for treatment of both conditions. Second, the accumulation of senescent immune cells, either macrophages or T cells (immune ageing) and their SASP link obesity to metaflammation[4] and chronic, low-grade, systemic inflammatory state (inflammaging), which contribute to ageing and age-related diseases[2,3] (FIG. 1). Moreover, immunity, metabolism, adipose tissue and muscle are highly integrated factors in ageing. Early accumulation of immune senescent cells seems to be a crucial event linking nutritional stress, chronic inflammation, obesity, ageing and age-related diseases (FIG. 1). In this scenario, changes in gut microbiota composition could be another early and/or causal mechanism, rather than a simple consequence of nutrient-rich diets, and could also be an intervention target. Furthermore, the two major pillars to counteract obesity and ageing are in fact the same — appropriate nutrition or dietary habits and physical exercise — and their beneficial

Key advances

- The visceral adipose tissue (VAT) of mice fed a diet of fast-food accumulates pro-inflammatory senescent cells that are cleared by physical exercise[5]
- The VAT of mice fed a high-fat diet accumulates a particular subset of senescent CD4$^+$ T cells that can induce obesity when transferred to lean mice[6]
- Human senescent fibroblasts implanted into immunodeficient mice induce senescence in macrophages that can be cleared by a bisphosphonate drug (clodronate), which kills phagocytic cells[7]
- 2 years of moderate calorie restriction induce a significant loss of body weight and a decrease in inflammatory markers without any detrimental effect on immune responses in humans[8]
- A short-term, high-fat, high-sucrose diet in Sprague Dawley rats quickly induces intramuscular fat deposition, fibrosis and increased number of pro-inflammatory cells, which are associated with early systemic inflammation and dynamic alterations in gut microbiota composition[9]

effects target basic biological mechanisms such as cell senescence and inflammation. Remarkably, the potential of calorie restriction, the most studied anti-ageing strategy, to reduce the accumulation of senescent cells has been largely neglected both in animal models and humans.

Based on these results, the biological role of senescent cells and SASP is even more complex and intriguing than previously thought. Senescent cells accumulate in tissues and organs during ageing, and data in mice indicate that their clearance can extend median lifespan and attenuate age-related deterioration of several organs[2,5]. In adult and old animals, senescent cells have a negative influence on health because they damage neighbouring cells via SASP and contribute to age-related degeneration and inflammation, that is, inflammaging. The gut microbiota, which undergoes profound changes and remodelling with age and is also quickly gaining the stage, is highly sensitive to diet and lifestyle and can be modified by prebiotics and probiotics[10]. Time will tell if, and to what extent, changes in human gut microbiota are causally related to obesity via cellular or macrophage senescence; this is a hot topic for research, together with the role of other cell types (such as tissue-resident natural killer cells whose main function is to sense cell alterations due to infections, transformation or exposure to other stressors) and their epigenetic changes in metaflammation. I look forward to seeing these issues clarified in 2017 and beyond.

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The author declares no competing interests.

GUT–BRAIN AXIS IN 2016

Brain–gut–microbiota axis — mood, metabolism and behaviour

Timothy G. Dinan and John F. Cryan

In 2016, key studies have increased our understanding of the part played by the brain–gut–microbiota axis in disorders as diverse as depression, obesity and autism spectrum disorder. The data indicate that alterations in gut-microbial composition can substantially affect central physiology, and that transplantation of the gut microbiota can transfer a behavioural or physiological phenotype.

The association of alterations in gut–brain interactions with functional bowel disorders, chronic abdominal pain syndromes and even eating disorders has become increasingly clear in the past few years. Modulation of gut–brain-axis function is associated with alterations in the stress response and overall behaviour in both animal models and in humans[1]. A high comorbidity exists between stress-related mental symptoms such as anxiety and IBS, a fact that has provided the greatest impetus for research into the importance of the gut–brain axis[2]. Over 50% of patients with IBS have comorbid depression or anxiety. Modulation of the gut–brain axis is increasingly being proposed as an appropriate target for the development of novel treatments for a wide variety of disorders that range from depression and anxiety to IBS, obesity and neurodevelopmental disorders[3].

The gut microbiota interact with the host through immune, neuroendocrine and neural pathways[4]. These pathways are components of the brain–gut–microbiota axis and preclinical evidence suggests that the microbiota can recruit this bidirectional communication network to modulate brain development, function and even behaviour. Preliminary studies have shown differences in the composition of the gut microbiota in patients with depression compared with healthy individuals. In one series of experiments, germ-free mice (but not conventionally raised animals) were protected from depression-like immobility induced by the forced-swim test as well as from anxiety behaviours. In addition, faecal samples from 58 Chinese patients with major depression and 63 healthy individuals as controls showed distinct differences in microbial composition[5]. In the patients with depression, the microbiota had alterations in species belonging to three bacterial phyla: Firmicutes, Actinobacteria and Bacteroidetes. Transplantation of faecal samples pooled from five patients with depression into germ-free mice resulted in depressive behaviour patterns; by contrast, faecal transplantation from five healthy individuals had no behavioural effect. Mice receiving microbiota from patients with depression showed disturbances in hippocampal gene activation and also in carbohydrate and amino-acid metabolism[5]. This study provides convincing evidence that the depressive phenotype can be transferred by transplantation of the microbiota. However, germ-free mice have abnormal immune-system development and brain anomalies (such as decreased levels of 5-hydroxytryptamine and brain-derived neurotrophic factor, changes in neuronal morphology in the amygdala, increased adult hippocampal neurogenesis and increased prefrontal cortical myelination[6]), which raises the question: would transplantation of microbiota from a patient with depression have a similar effect if the immune system and brain development in the recipient animal were normal?

To answer this question, Kelly and colleagues[7] recruited 34 patients with major depression and 33 healthy individuals matched for age and sex. Plasma levels of cytokines, C-reactive protein, salivary cortisol and plasma lipopolysaccharide-binding protein were determined by ELISA, and showed alterations supporting a proinflamatory phenotype associated with depression. Plasma levels of tryptophan and kynurenine and composition of faecal microbiota were also determined. Depression was associated with decreased gut-microbiota richness and diversity. A faecal microbiota transplant was prepared from a subgroup of patients with depression or from healthy individuals and transferred by oral gavage to a microbiota-deficient rat model. The model comprised normal rats with a developed immune system and normal brain function that were given a cocktail of antibiotics to eliminate the gut microbiota. Transplantation

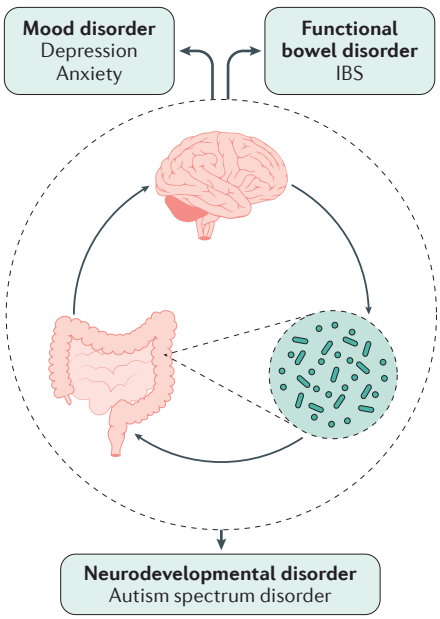

Figure 1 | **The brain–gut–microbiota axis.** The gut microbiota is altered not only in functional bowel disorders such as IBS but in mood disorders such as depression, neurodevelopmental disorders such as autism spectrum disorder and in metabolic disorders.

Key advances

- Transplantation of microbiota from patients with depression to microbiota-depleted animals induces behavioural and physiological features characteristic of depression in the recipient animals[7]
- Increased production of acetate by an altered gut microbiota in mice and rats leads to activation of the parasympathetic nervous system, which in turn promotes increased glucose-stimulated insulin secretion, increased ghrelin secretion, hyperphagia and obesity[8]
- A maternal high-fat diet induces changes in the social behaviour of offspring and is linked to gut microbiota alterations and anomalies in the mesolimbic dopamine reward system; treatment with the commensal *Lactobacillus reuteri* reverses the deficits in social behaviours[10]

of microbiota from patients with depression to microbiota-depleted rats induced behavioural and physiological features characteristic of depression in the recipient animals, including anhedonia, anxiety-like behaviours and alterations in tryptophan metabolism[7]. This study provides further evidence that the gut microbiota have a causal role in the development of features of depression and could provide a tractable target in the treatment and prevention of this disorder.

Several lines of evidence suggest that brain function and behaviour are influenced by microbial metabolites. Short-chain fatty acids (SCFAs), such as butyrate, propionate and acetate, are key products of the gut microbiota. Although no direct evidence currently exists that SCFAs travel via the blood stream to the brain in humans, findings increasingly support the indirect actions of SCFAs. For example, increased production of acetate by an altered gut microbiota in mice and rats leads to activation of the parasympathetic nervous system, which in turn promotes increased glucose-stimulated insulin secretion (GSIS), increased ghrelin secretion, hyperphagia, obesity and related sequelae[8]. Perry and colleagues[8] found that, in contrast to propionate and butyrate, whole-body turnover of acetate, and concentrations of acetate in the plasma and faeces, were markedly increased in insulin-resistant rats after a high-fat diet (HFD) compared with chow-fed rats[8]. Acetate infusion in chow-fed rats strongly replicated the increases in GSIS measured in HFD-fed rats, implicating heightened acetate turnover in driving the increases in GSIS in HFD-fed rats. By contrast, supplementing butyrate in chow-fed rats to match the turnover rates observed in HFD-fed rats surprisingly had no effect on GSIS. To examine the role of the gut microbiota in acetate-induced hyperinsulinaemia, HFD-fed rats were treated with broad-spectrum, nonabsorbable oral antibiotics, which resulted in a major reduction in GSIS during a hyperglycaemic clamp. Faecal transplantations transferred the acetate turnover, faecal acetate and GSIS associated with the donor animal to the recipient animal. These data suggest that the gut microbiota are the source of most of the increase in endogenous acetate production in HFD-fed rats. When the vagus nerve was severed in the rats, infusion with acetate exhibited a marked reduction in plasma insulin concentrations throughout a hyperglycaemic clamp, without any change in plasma glucagon concentrations, when compared with rats that had an intact vagus nerve[8]. Overall, these data support the view that increased acetate production resulting from a nutrient–gut-microbiota interaction and subsequent parasympathetic activation is a possible therapeutic target for obesity.

> “…the microbiota can recruit this bidirectional communication network to modulate brain development, function and even behaviour”

Maternal obesity has been linked with neurodevelopmental disorders in offspring, including autism spectrum disorder[9]. Now, Buffington and colleagues[10] have shown that obesity in mice induced by a maternal high-fat diet (MHFD) is associated with social behavioural deficits, which are mediated by alterations in the offspring gut microbiota. The diversity of the microbiota in MHFD offspring was reduced compared with animals on a regular diet, with an especially notable reduction in *Lactobacillus* spp. The MHFD-induced changes in the offspring gut microbiota were associated with major alterations in the mesolimbic dopamine reward system within the ventral tegmental area. Furthermore, treatment with a commensal bacterial species *Lactobacillus reuteri* increased levels of oxytocin (known to increase social behaviour), ameliorated synaptic dysfunction in the ventral tegmental area and selectively reversed social deficits in MHFD offspring. This amelioration of the deficient social behaviour was entirely specific to *L. reuteri*, as treatment with another *Lactobacillus* species, *Lactobacillus johnsonii,* whose abundance is also reduced in the gut microbiota of MHFD offspring, failed to normalise social behavioural deficits. These data support previous evidence suggesting a role of the gut microbiota in social behaviour[10].

Understanding the way in which the gut microbiota influence gut–brain-axis communication (FIG. 1) has been the subject of considerable research effort in the past few years. The gut microbiota are now recognized to influence processes such as the stress response and, consequently, to play a part in the pathophysiology of functional bowel disorders such as IBS[2]. Whether changes in the microbiota are central to the pathophysiology of at least some psychiatric disorders such as depression has yet to be definitively demonstrated, although the studies published this year provide further support for such a view. Increasingly, evidence is accumulating for a role of the gut microbiota in autism spectrum disorder and the negative metabolic consequences in obesity. Future studies must determine whether exciting new data, which has largely been obtained from preclinical animal experiments, translates to humans; only time will tell.

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Competing interests statement
The authors declare no competing interests.

HBV IN 2016

Global and immunotherapeutic insights into hepatitis B

Mala K. Maini and Antonio Bertoletti

The burden of HBV infection remains high and new strategies to improve HBV vaccination and therapy are needed. Key research in 2016 highlights the efficacy of current approaches and proposes new concepts for some of the immunological defects that need to be overcome for HBV functional cure.

HBV is a hepatotropic infection that is a leading global cause of viral hepatitis. HBV is non-cytopathic but chronic infection can trigger immunopathology, leading to chronic liver inflammation and fibrosis, ultimately resulting in liver cirrhosis and/or hepatocellular carcinoma (HCC). Work by Stanaway *et al.*[1] published in 2016 has highlighted how widespread viral hepatitis and its complications remain in the world today, with the burden of death and disability increasing at an alarming rate. Although most other communicable diseases are decreasing, the absolute burden and relative rank of viral hepatitis is increasing. Viral hepatitis is now the seventh leading cause of death worldwide, with attributable deaths increasing from an estimated 0.89 million in 1990 (95% uncertainty interval 0.86–0.94) to 1.45 (95% uncertainty interval 1.38–1.54) million in 2013.

Preventive measures remain of paramount importance for HBV and HCV control, including the prophylactic HBV vaccine that is now part of universal immunization programmes in many countries worldwide. An important demonstration of how this approach reduces long-term morbidity from HBV infection comes from a 2016 study in Taiwan (REF. 2). Chang *et al.*[2] analysed 1,509 patients with HCC according to whether they were born before or after the stepwise introduction of HBV immunization of newborn babies in 1984. Adjusting for age, a significant reduction in HCC rates was shown in vaccinated birth cohorts reaching adulthood (from 0.92 to 0.23 HCC incidence per 100,000 person-years), in line with previous findings in children and adolescents. The most common reason for breakthrough infection in vaccinated infants was high-level hepatitis B 'e' antigen (HBeAg) infection in the mother. An additional strategy to tackle such transmission was evaluated by Pan *et al.*[3] who randomly assigned 300 $HBeAg^+$ mothers with >200,000 IU/ml of HBV DNA in the third trimester of pregnancy to receive tenofovir from week 30 or 32 to 4 weeks postpartum or not, in addition to standard immunoprophylaxis of the infant. Tenofovir substantially reduced mother-to-child transmission in this high-risk group, although it did not eliminate it completely (HBV infection incidence dropped from 18% to 5% of infants). Importantly, birth defects were not increased in the tenofovir group, in line with its good safety record in HIV infection in pregnancy.

The improved efficacy of prophylactic strategies should ultimately reduce the prevalence of HBV over the coming decades but a huge burden of chronic disease currently remains. In contrast to the astonishing efficacy of new HCV drugs, current therapies can efficiently suppress HBV replication and reduce liver inflammation, but are unable to clear the virus or even achieve sustained virologic control after therapy withdrawal. Exciting progress has been made in preclinical development of new classes of antivirals for HBV (not discussed here), but harnessing the immune system is probably still required for effective off-treatment responses. Encouragingly, the immune system has the inherent capacity to maintain HBV under effective long-term control in the majority of infected adults who resolve infection, whereas chronic hepatitis B (CHB) is characterized by profound defects of HBV-specific antiviral immunity. Attempts to boost such immunity with immune-based therapies have, to date, yielded disappointing results. This issue is exemplified by the failure of a therapeutic vaccine to induce any substantial reductions in hepatitis B surface antigen (HBsAg) in a phase II trial of 178 patients with CHB already immunosuppressed on antiviral agents[4], despite induction of HBV-specific T cells observed with this vaccine in healthy volunteers in a phase I trial. This heat-inactivated, yeast-based vaccine containing HBsAg, hepatitis B core antigen (HBcAg) and hepatitis B x antigen (HBxAg) also achieved limited boosting of HBV-specific T-cell responses in patients with CHB in the phase II trial, but notably not against HBsAg[4].

To achieve an off-treatment 'functional cure', we require improved understanding of factors limiting the development of efficient antiviral immunity in different disease phases of CHB. In 2016, two interesting papers using a mouse model of HBV persistence[5,6] described new potential mechanisms of HBV-specific T-cell tolerance, while a third paper[7] provided evidence that such tolerance is not fully operational in the early phases of CHB. Tian *et al.*[5] investigated immunological mechanisms contributing to the propensity of HBV to establish chronicity after vertical infection (rather than the typical control seen after adult horizontal acquisition[1]).

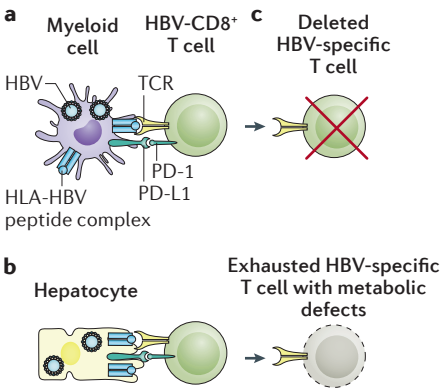

Figure 1 | **Presentation of HBV antigens by myeloid cells or hepatocytes triggers HBV-specific T-cell deletion or inactivation.** **a** | Myeloid cells presenting HBV antigens and overexpressing PD-L1 alter the functionality of HBV-specific $CD8^+$ T cells. **b** | HBV antigen presentation by hepatocytes can also induce deletion or functional inactivation of T cells. **c** | T cells in patients with chronic hepatitis B are deleted or functionally exhausted and display metabolic modifications.

Key advances

- Vertical HBV transmission from HBeAg$^+$ mothers to newborn babies can be safely reduced by tenofovir treatment in the last trimester of pregnancy[3]
- Presentation of HBV viral antigens by hepatic macrophages represents, in HBV animal models, an important mechanism of HBV-specific T-cell tolerance[5,6]
- Evidence of liver damage, HBV-specific T-cell responses and pro-oncogenic events in patients during the early phase of chronic hepatitis B challenge the concept of complete immunotolerance[8]
- Molecular characterization of exhausted HBV-specific T cells from patients with chronic hepatitis B reveals the presence of metabolic and mitochondrial dysfunction[9]

Quantitative and qualitative defects of HBV-specific CD8$^+$ T cells present in mice born to mothers with transgenic expression of HBeAg rendered them unable to clear HBV from the liver following hydrodynamic transfection. Further experiments suggested that HBeAg-conditioned macrophages were indispensable for HBV-specific CD8$^+$ T-cell tolerance induction, through their expression of the co-inhibitory ligand PD-L1.

Another study proposed that presentation of HBV viral antigens by hepatic macrophages represents an important mechanism of HBV-specific T-cell tolerance induction[6]. In the same mouse model of hydrodynamic HBV infection, HBV-specific CD4$^+$ T-cell deletion was caused by their intrahepatic retention mediated by chemokines produced by intrahepatic macrophages[6]. Here, T-cell IFNγ (classically considered important for HBV control) induced hepatric macrophages to secrete chemokines and paradoxically drove HBV-specific T-cell deletion by intrahepatic trapping and deletion.

Both these studies indicate that HBV-specific T-cell deletion might be caused directly by persistent presentation of viral antigens by infected hepatocytes, and by cross-presentation of HBV antigens by myeloid cells present in the liver (FIG. 1), highlighting this cell type as a future therapeutic target. These interesting results must be interpreted with caution because of the inherent limitations of the hydrodynamic mouse model to fully recapitulate the complexity of natural HBV infection; discrepancy between data obtained in natural infection and mouse models has been reported[7].

The issue of HBV immunotolerance was further explored in 2016 in patient samples[8]. The level of HBV-DNA integration, clonal hepatocyte expansion and HBV-specific T-cell response were similar in CHB, irrespective of clinical and virological phase. Liver hepatocyte turnover and HBV-DNA integration were present in young (14–30 years) 'immunotolerant' patients, showing that liver damage and potential pro-oncogenic events already occur in this first phase of CHB. As the level of HBV-specific T-cell response was similar in patients categorized as immunotolerant or immune active, the researchers argued that this terminology seems inadequate to define the clinical and immunological features of the heterogeneous populations of patients with CHB. That the degree of HBV-specific immune tolerance is not linked to age or levels of liver inflammation has important therapeutic consequences as the field starts to select patient groups for new immune-based therapies.

The universally weak HBV-specific T-cell responses observed in all stages of CHB underscore the need to further define underlying molecular defects. One such defect has been found in mitochondria of HBV-specific T cells, which have defective depolarization[9] (FIG. 1). The capacity of T cells to flexibly metabolize glucose and other nutrients has been identified as one critical determinant shaping immune function. In this study of patients with CHB, whereas functional cytomegalovirus-specific CD8$^+$ T cells could utilize oxidative phosphorylation in the absence of glucose, the exhausted PD-1$^+$ HBV-specific CD8$^+$ T cells within the same donors were heavily dependent on glucose for glycolysis. The possibility of targeting mitochondrial and or metabolic defects as novel therapeutic approaches was exemplified by the capacity of IL-12 to recover mitochondrial potential and oxidative phosphorylation of HBV-specific T cells *in vitro*.

Dissecting the extent of immunological defects present in heterogeneous CHB patient populations will be necessary to tailor distinct immunotherapeutic strategies. Such immunological information must be coupled with virological data that goes beyond the current quantification of overall serum HBV level to instead analyse the composition of infected hepatocytes. A detailed analysis using novel immunohistochemical techniques[10] showed that HBV-infected hepatocytes during CHB do not constitute a homogeneous population of infected targets, but are a complex mosaic of cells expressing different levels of HBV antigens and DNA. In particular, the demonstration of a mutually exclusive pattern of expression of HBsAg and whole HBV DNA in infected hepatocytes constitutes a surprising finding that needs to be considered when assessing novel immunological approaches to deal with the complex interactions between HBV and its natural host.

The task of achieving a functional cure for the still large population of chronically infected patients remains challenging. Nevertheless, better understanding of the mechanisms of HBV immunotolerance will improve the success of future therapies that will probably involve combined antiviral and immune strategies. Understanding how best to combine these different approaches and select the most appropriate patient populations to treat will be the next major challenges.

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LIQUID BIOPSY IN 2016

Circulating tumour cells and cell-free DNA in gastrointestinal cancer

Klaus Pantel and Catherine Alix-Panabières

The challenge to obtain needle biopsy samples from patients with cancer has steered the development of new blood-based diagnostics called 'liquid biopsy'. In 2016, major advances have been made in the use of circulating tumour cells and cell-free DNA for monitoring tumour evolution in patients with cancer of the gastrointestinal tract, with a focus on colorectal cancer.

The term 'liquid biopsy' was originally introduced 6 years ago to define the analysis of circulating tumour cells (CTCs) in the blood of patients with cancer[1], but it has now been extended to the examination of circulating cell-free DNA (ctDNA), microRNAs, microvesicles and even platelets, which all contain tumour-derived information[2,3]. In particular, analyses of CTCs and ctDNA have paved new diagnostic avenues[4]. Key clinical applications include the detection of cancer, prediction of prognosis in patients with curable disease, monitoring of systemic therapies and stratification of patients based on the detection of therapeutic targets or resistance mechanisms[4] (FIG. 1).

Tumour heterogeneity is a hallmark of cancer[5] that also offers an important rationale for blood-based analysis as compared to single tissue. Russo *et al.*[6] showed in a study published in 2016 how genomic heterogeneity is associated with acquired resistance to targeted agents. Studying EGFR blockade in colorectal cancer (CRC), the authors showed that liquid biopsies could be integrated with radiological imaging to monitor the effect of individual oncogenic alterations on lesion-specific responses. Biopsy of a patient's progressing liver metastasis following prolonged response to cetuximab revealed a MEK1 mutation (Lys57Thr) as a novel mechanism of acquired resistance. This lesion regressed upon treatment with panitumumab and the MEK inhibitor trametinib. In ctDNA, a previously unrecognized KRAS mutation (Gln61His) was identified that increased despite therapy, and this same *KRAS* mutation was later found in a separate non-responding metastasis. Thus, separate tumour lesions in the same patient can harbour distinct mutations that are detectable by liquid biopsy. Analysis of a single-lesion biopsy might therefore be inadequate to guide selection of subsequent targeted therapies.

Most literature on ctDNA is derived from the analysis of patients at advanced disease stages, when the yield of ctDNA is much higher than in earlier stages. In patients with cancer, detection of minimal residual disease by monitoring of peripheral blood after surgery has become the next frontier. In this context, Tie *et al.*[7] described promising findings of ctDNA monitoring in patients with stage II CRC. Thus far, it is difficult to determine which of these tumours will recur and to identify patients who would benefit from adjuvant chemotherapy after surgery. Tie *et al.*[7] analysed 1,046 plasma samples from a prospective cohort of 230 patients with resected stage II colon cancer and showed that ctDNA can persist in a patient's blood after surgery. Most importantly, this persistence was associated with an increased risk of relapse. Similar prognostic value in patients with stage II CRC has been already reported for CTCs detected at surgery. Future interventional studies are now required to evaluate whether this group of high-risk patients with stage II CRC will profit from chemotherapy to prevent recurrence.

An important clinical application of the liquid biopsy is the early evaluation of therapy efficacy. In this setting, Li *et al.*[8] prospectively evaluated 136 patients with an advanced gastric cancer, counting CTCs using the FDA-cleared CellSearch system (Janssen Diagnostics, USA) at baseline and after 6 weeks of chemotherapy. The results of this study, published in 2016, showed clearly that unfavourable post-treatment CTC levels (≥3 CTCs per 7.5 ml blood) were correlated with a poor therapeutic outcome reflecting an ineffective therapeutic response. Specifically, conversion to a favourable CTC level following therapy improved prognosis, but patients who changed to an unfavourable CTC level had a worse prognosis. CTCs are defined as an independent biomarker predictor of decreased progression-free survival and overall survival in patients with advanced gastric cancer. This

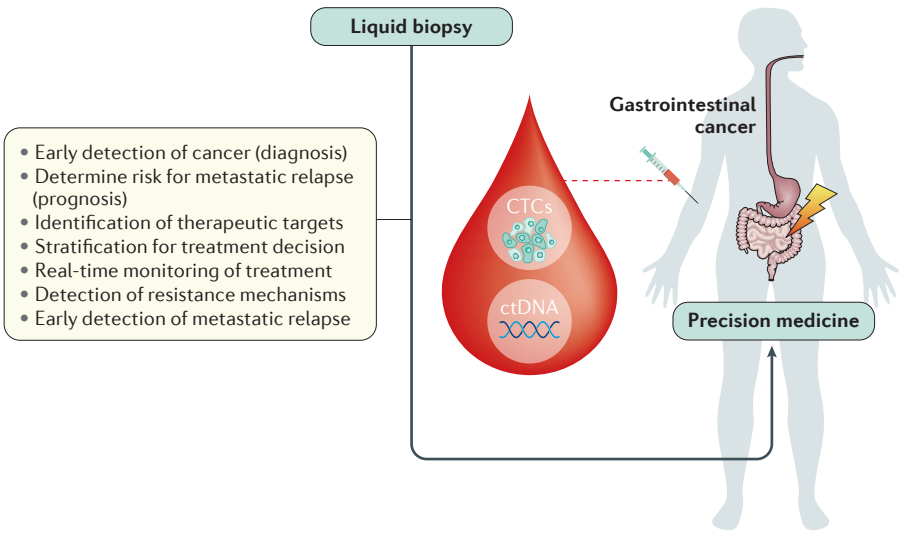

Figure 1 | **Key applications of circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA) as liquid biopsies for precision medicine.** Blood can be sampled repeatedly to detect cancer, predict risk for metastatic relapse, identify therapeutic targets, stratify patients to the most effective drugs, monitor the efficacy of therapies (for example, persistence or increased levels of CTCs and/or ctDNA indicates resistance to therapy), detect potential resistance mechanisms and detect early relapse in patients without metastatic disease or the progression of metastases.

Key advances

- Genomic heterogeneity in colorectal canceris associated with acquired resistance to targeted agents[6]
- Circulating DNA persistence after colon cancer surgery is associated with an increased risk of relapse[7]
- Dynamic monitoring of circulating tumour cells (CTCs) evaluates therapeutic efficacy in advanced gastric cancer[8]
- Metastasis-competent colon CTCs (CTC-MCC-41) display a specific transcription programme with upregulation of key genes related to energy metabolism, DNA repair and stemness[10]

study demonstrated that dynamic monitoring of CTCs evaluated therapeutic efficacy in advanced gastric cancer, and these changes in CTC count over time might help in quickly identifying inefficient treatments.

Understanding the molecular mechanisms that regulate the biology of metastasis-competent CTCs is of utmost importance in unravelling the formation of metastases and tumour relapse in patients with cancer. Cayrefourcq *et al.*[9] derived the first cell line (CTC-MCC-41) from CTCs isolated from the blood of a patient with colon cancer. Subsequently, in 2016, Alix-Panabières *et al.*[10] determined the molecular bases underlying differences between this colon CTC line and well-described cancer cell lines derived from primary tumours and from metastatic sites[10]. The results showed clearly that the CTCs displayed a very specific transcription programme. Interestingly, among the 1,624 transcripts exclusively upregulated in CTC-MCC-41 cells, key genes related to energy metabolism, DNA repair and stemness were observed. Such data might supply insights for the discovery of new pathways and biomarkers to identify the most aggressive CTC subpopulations with stem cell properties. CTC lines could contribute to the development of new drugs to eradicate metastasis-initiator CTCs causing relapses and cancer-related death in individuals with cancer.

In conclusion, CTCs and ctDNA analyses are complementary, depending on the context of use. Prediction of prognosis in patients with potentially curable disease can already be achieved and might lead to improved tumour staging. Monitoring chemotherapy or other targeted therapies by longitudinal measurements of CTCs or ctDNA is feasible and the consequences are currently being investigated in clinical trials. Interventional studies on treatment stratification based on the detection and molecular characterization of CTCs and ctDNA have started and should be the focus to implement liquid biopsy into precision medicine. New promising analytes of liquid biopsy (for example, cell-free microRNA, microvesicles or platelets) should be further explored and validated (such as within the European Innovative Medicines Initiative consortium project, CANCER-ID).

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Competing interests statement
The authors declare no competing interests.

IBD IN 2016

Biologicals and biosimilars in IBD — the road to personalized treatment

Krisztina B. Gecse and Péter L. Lakatos

In 2016, personalized medicine for IBD has been evolving. Increasing comfort with biosimilar infliximab was achieved with 'real-life' data. Drugs with alternative modes of action confirmed substantial benefit, even in patients failing anti-TNF agents. Adipose-derived mesenchymal stem cells yielded a new treatment option for perianal fistulas.

Crohn's disease and ulcerative colitis are classified as chronic inflammatory bowel diseases characterized by intermittent disease flares and periods of remission. In the absence of adequate treatment, chronic inflammation results in irreversible intestinal damage and disability. The introduction of anti-TNF agents led to a paradigm shift in management strategies; infliximab and adalimumab induce and maintain clinical remission, as well as mucosal healing. However, one-third of patients with IBD do not respond to anti-TNF treatment and up to 40% of initial responders lose response or develop intolerance over a year[1]. Thus, optimization of anti-TNF treatment is essential and drugs with new modes of action are needed.

Correlation between serum anti-TNF levels and clinical, as well as endoscopic, outcomes has been previously demonstrated. However, patients with optimal serum drug concentrations can also fail to respond to treatment. Moreover, clinical and endoscopic response also correlates with the number of TNF-expressing immune cells, as detected by confocal laser endomicroscopy. To shed further light on the missing links of the pharmacokinetics of biologic agents, Yarur *et al.*[2]

measured intestinal tissue TNF levels as well as tissue and serum drug concentrations in a cross-sectional study of 30 patients with IBD who had varying degrees of disease severity and were treated with infliximab or adalimumab. Inflamed intestinal tissues exhibited markedly higher anti-TNF drug levels than matched uninflamed samples, which correlated with the increased TNF levels of inflamed tissues, as well as with the severity of inflammation. However, in moderate to severe inflammation, the drug to TNF concentration ratio in tissue was lower than in mild inflammation or in the absence of inflammation. Accordingly, these patients were more likely to exhibit a high serum and a low tissue anti-TNF concentration, which was interpreted as a so-called serum to tissue anti-TNF mismatch. These findings suggest that primary non-responders to anti-TNF treatment who exhibit moderate to severe inflammation might lack sufficient drug tissue levels to neutralize TNF production. A plausible explanation is that severe inflammation leads to increased faecal loss of anti-TNF agents, which was previously associated with primary non-response in severe ulcerative colitis[3]. The advances in understanding pharmacokinetics of biologic agents are crucial to optimize patient management.

The reversed engineering process, the novel legal framework and the lack of clinical data in IBD initially led to considerable concern after the first infliximab biosimilar received marketing authorization from the European Medicines Agency in 2013 for all indications of the originator product. In the largest, prospective, nationwide observational cohort 210 consecutive patients with IBD were treated with CT-P13, a biosimilar of infliximab[4]. Biosimilar infliximab was effective in inducing clinical response and remission at week 14 in both Crohn's disease and in ulcerative colitis. This effect was maintained until week 30 and was associated with a substantial decrease in levels of inflammatory biomarkers, such as C-reactive protein level (21 mg/l and 32 mg/l at baseline, which decreased to 11 mg/l and 7.5 mg/l at week 14 in Crohn's disease ($P = 0.02$) and ulcerative colitis ($P = <0.001$), respectively). Until week 30, adverse events occurred in 17% of patients. Infusion reactions occurred in 7% and infectious adverse events occurred in 6% of patients. Additionally, clinical remission rates were markedly higher in infliximab-naive patients than those with prior exposure to the originator compound. Accordingly, these patients exhibited substantially higher baseline anti-drug antibody positivity than infliximab-naive patients[4]. Notably, a substantial proportion of patients with infusion reactions had previously received the originator infliximab. In line with this finding, Ben-Horin *et al.*[5] showed that antibodies against the originator drug Remicade (Janssen, USA) from treated patients with IBD recognized, crossreacted and neutralized the TNF-binding capacity of the biosimilar Remsima (Celltrion, South Korea). This crossreactivity of Remicade anti-drug antibodies and Remsima suggested similar immunodominant epitopes and immunogenic potentials of the biosimilar and originator molecules.

NOR-SWITCH is a randomized, double-blind, parallel-group study that evaluated switching from the innovator infliximab to biosimilar infliximab compared with continued treatment with the innovator compound[6]. Across all indications, 481 patients were included in the study. Disease worsening during the 52-week study period occurred in 26.2% of those who remained on the originator and in 29.6% of those who were switched to the biosimilar. This percentage difference fell within the pre-specified non-inferiority margin and disease worsening was comparable among indications. On the basis of these results, the efficacy and safety of CT-P13 proved comparable to the originator compound as reported by earlier observational studies. In addition, efficacy was maintained after switching from the originator to the biosimilar.

Vedolizumab inhibits interaction between α4β7 integrin and mucosal addressin cell adhesion molecule 1 to prevent the transmigration of gut-homing T cells into the inflamed intestinal tissue. Phase III trials previously showed its efficacy in moderate to severe Crohn's disease and ulcerative colitis. Thus, vedolizumab was the first drug to receive regulatory approval representing a new, gut-selective mode of action in the IBD treatment armamentarium. Efficacy and safety of vedolizumab induction treatment was confirmed by Amiot *et al.*[7], who analysed a real-life cohort of patients in a compassionate early access programme for those who failed TNF antagonists. 173 patients with Crohn's disease and 121 patients with ulcerative colitis received vedolizumab 300 mg intravenously at weeks 0, 2, and 6, and then every 8 weeks. At week 14, steroid-free clinical remission was achieved in 31% of patients with Crohn's disease and 36% of patients with ulcerative colitis, and clinical response was obtained in 51% and 50%, respectively. Adverse events occurred in 32% of patients, of with 5% discontinuing treatment.

Ustekinumab is a human monoclonal antibody against the p40 subunit of IL-12 and IL-23. In a phase IIb study, intravenous ustekinumab showed notable benefit in terms of clinical response in moderate to severe Crohn's disease refractory to TNF antagonists and subcutaneous doses were effective during a 22-week maintenance period. In November 2016, Feagan *et al.*[8] reported the results of the phase III trial, which consisted of two 8-week induction studies and a maintenance trial for patients with moderate to severe Crohn's disease. UNITI-1 included patients who had failed anti-TNF therapy and UNITI-2 recruited those who were refractory to conventional treatment. Patients were randomly assigned to receive a single infusion of ustekinumab (130 mg or 6 mg/kg) or placebo. Already at week 6, intravenous ustekinumab induced markedly higher rate of clinical response than placebo in both induction studies (in UNITI-1 34.3%, 33.7%, and 21.5%, respectively, $P \leq 0.003$; in UNITI-2, 51.7%, 55.5%, and 28.7%, respectively, $P < 0.001$). Among patients with response to induction treatment, clinical remission at week 44 was

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Key advances

- Patients with IBD and moderate to severe inflammation are more likely to exhibit a 'serum to tissue anti-TNF mismatch', which might account for primary non-response[2]
- 'Real-life' experience with infliximab biosimilars suggests that CT-P13 is effective and safe in the induction and maintenance of clinical remission and response in IBD[4]; efficacy of one-time switching from the originator to the biosimilar is non-inferior compared with continued originator treatment[6]
- Ustekinumab was more effective than placebo in inducing clinical response and maintaining clinical remission in moderate to severe Crohn's disease in a phase III trial[8]; in a real-life cohort, vedolizumab showed clinical benefit in half of the patients with IBD who had failed anti-TNF treatment[7]
- Local treatment with Cx601 stem cells is yielding a novel therapeutic option for refractory perianal Crohn's disease fistulas[10]

substantially higher in patients receiving subcutaneous maintenance ustekinumab (90 mg every 8 or 12 weeks) than placebo (53.1%, 48.8% and 35.9%, $P = 0.005$ and $P = 0.04$, respectively). The rate of adverse events was similar in the ustekinumab-treated and placebo-treated groups and was consistent with the cumulative data from its indication for psoriasis. Additionally, Wils *et al.*[9] reported on the efficacy of compassionate use of subcutaneous ustekinumab in 122 consecutive patients with Crohn's disease who had failed anti-TNF treatment. At 3 months, two-thirds of patients exhibited clinical benefit, with normalization of C-reactive protein levels occurring in 41% and endoscopic response obtained in 77% of patients. There has been a long-term need for drugs with new modes of action for the growing number of patients refractory to anti-TNF agents and major advances have been made to meet this need in 2016.

Current treatments options for fistulizing Crohn's disease are limited by quality of evidence and long-term efficacy but results from Panés *et al.*[10] yielded a new treatment option for perianal Crohn's disease fistulas. In a phase III, randomized, double-blind, parallel-group study, 212 patients with treatment refractory complex perianal fistulas were randomly assigned to receive intralesional injection of allogeneic, expanded, adipose-derived stem cells (Cx601, 120 million cells) or placebo. 50% of patients treated with Cx601 compared with 34% of the placebo group ($P = 0.024$) achieved the combined primary end point of clinical closure of all fistulas and absence of abscess on MRI at week 24. Thus, adipose-derived stem cells could open up a new, local treatment option for refractory perianal fistulas.

During 2016, the comfort with biosimilar use and acceptance of the concept of interchangeability undoubtedly increased. However, it is difficult to estimate the effect of multiple switches and long-term immunogenicity when numerous biosimilars of an agent become available. The latest advances and ongoing drug development with new modes of action might bring a new era in the treatment of IBD. Moreover, new tools to predict response to a specific treatment could further aid the personalized use of biological therapies in IBD.

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PRIMARY BILIARY CHOLANGITIS IN 2016

High-definition PBC: biology, models and therapeutic advances

Gwilym J. Webb and Gideon M. Hirschfield

In 2016, obeticholic acid became the first new licensed therapy for primary biliary cholangitis in >20 years. This therapeutic came at a time of improved disease understanding from biliary and immunological mechanistic insights.

Primary biliary cholangitis (PBC, formerly known as primary biliary cirrhosis) is an important but rare chronic immune-mediated liver disease. Globally, it is estimated that 1 in 1,000 women aged >40 years live with PBC and the risks of disease progression to cirrhosis and liver failure[1]. In 2016, our understanding of PBC pathogenesis has been be further refined on the basis of elegant basic and clinical science, including the concept of high-risk and low-risk disease, particularly as recognized after evaluation of treatment response to the first-line agent ursodeoxycholic acid[2]. Increasingly, it is now recognized that a multistep model of PBC pathogenesis can be identified (FIG. 1)[1]. In this setting, therapies for those stratified at high-risk of disease progression despite ursodeoxycholic acid use will be more or less effective, depending on when they are initiated and how they work[2].

Animal models of PBC have always been a struggle, as attempting to recapitulate such a complex interaction of immunological injury and localized tissue response is challenging.However, work from the Gershwin and Young groups in 2016 has made progress in developing a more meaningful disease model for study, and one that captures the gender distinction seen in PBC[3]. This group studied mice characterized by prolonged chronic expression of IFNγ. Interestingly, in this model, mice developed murine PBC characterized by female predominance, upregulation of total bile acids, spontaneous production of anti-mitochondrial antibodies and portal duct inflammation. Overall, this work highlighted the importance of interferon signalling in initial PBC pathogenesis. Inflammatory responses mediated by type 1 T helper cells have been recognized as critical for loss of immunological tolerance and

this data parallels an understanding of PBC genetic risk that spans key immune-regulatory pathways including IL-12 and JAK–STAT signalling[4], pathways potentially additionally modifiable by oestrogen exposure.

A dichotomous understanding of PBC has often been raised between the importance of immunological injury alongside cellular response to biliary injury and cholestasis. With more sophisticated experimental approaches, it seems that this distinction is beginning to blur and in a way that might indicate better therapeutic approaches. Notably, it has become relevant that the Cl^-/HCO_3^- exchanger (AE2; anion exchanger 2) and an intact biliary glycocalyx are important in maintaining a protective biliary HCO_3^- 'umbrella'. In 2016, Chang *et al.*[5] explored this understanding, demonstrating *in vitro* that soluble adenylyl cyclase, a bicarbonate sensor, regulates bile-salt-induced apoptosis in a manner dependent on intracellular Ca^{2+} stores, which are mediated by intrinsic apoptotic pathways. These findings suggest that downregulation of AE2 in PBC can sensitize cholangiocytes to apoptotic insults by activating soluble adenylyl cyclase. Intriguingly, in related work, Hisamoto *et al.*[6] found that hydrophobic bile acids (glycochenodeoxycholic acid) suppress expression of AE2 in biliary epithelial cells by inducing reactive oxygen species and enhancing biliary epithelial cell senescence, which induced bile duct inflammation. Reduced AE2 expression also upregulated the expression of CD40 and HLA-DR, as well as production of IL-6, IL-8 and CXCL10 from biliary epithelial cells in response to Toll-like receptor ligands (CXCL10 is secreted in response to IFNγ). This work equally highlights how changes to normal biliary epithelial cell physiology are relevant, not only to a response to injury, but also to subsequent signalling pathways leading to chronic inflammatory responses. Thus, the senescence-associated secretory phenotype of biliary epithelial cells might be relevant in PBC and other cholestatic liver diseases, and changes in AE2 function could be a 'bridge' between the immune system and cholestasis. Additionally, that IL-17-positive T cells are detected in portal infiltrates close to inflamed bile ducts expressing the CCR6 ligand CCL20 is already known[7]. Cytokine-treated human cholangiocytes secrete CCL20 and induce CCR6-dependent migration of type 17 T helper cells, suggesting that local cholangiocyte chemokine secretion then localises these pathogenic and damaging cells to bile ducts[7]. Intriguingly, *CCL20* is a recognized genetic risk locus for PBC development and could be a meaningful added target for intervention[4].

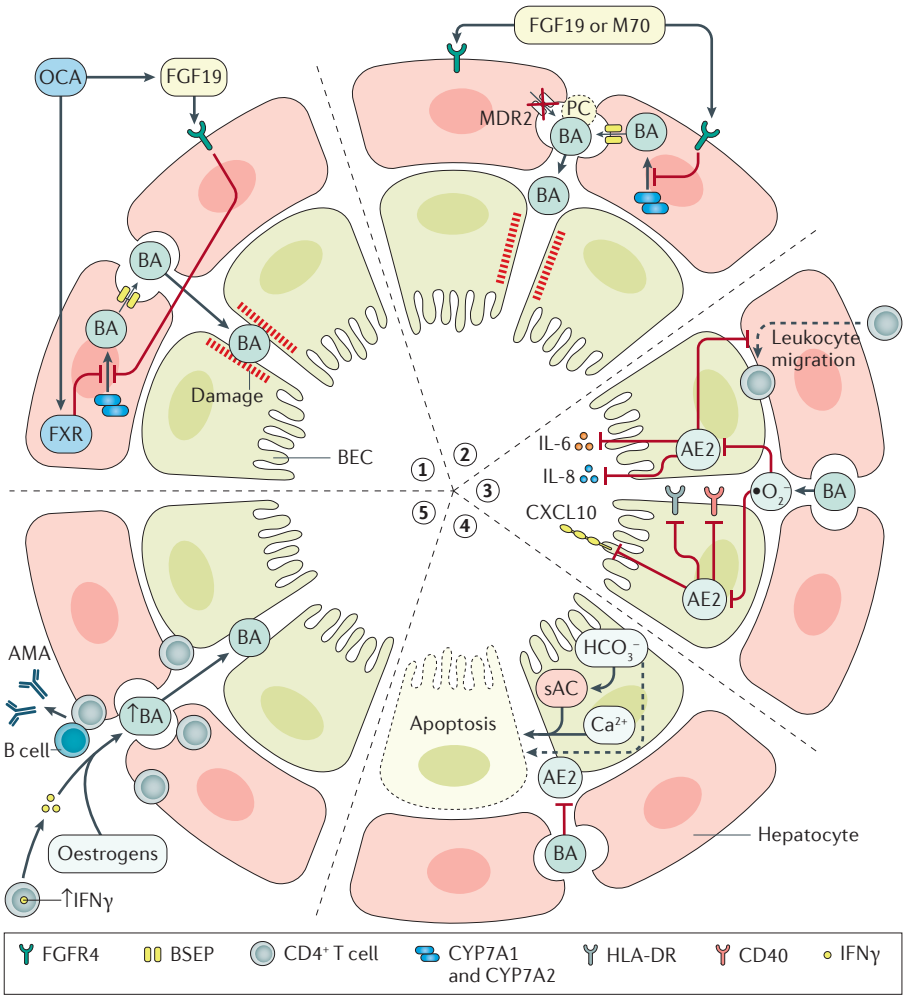

Figure 1 | **Converging themes in the pathophysiology and treatment of PBC.** Obeticholic acid (OCA) downregulates bile acid (BA) production and limits further damage to small bile ducts through inhibited expression of the bile acid synthesis enzymes CYP7A1 and CYP27A1, and through synthesis of FGF19 (REF. 10) (1). Mice with targeted disruption of MDR2 have unchaperoned bile acids, which are more reactive and cause progressive damage to biliary epithelial cells (BEC) and liver fibrosis; FGF19 and M70 act to reduce bile acid synthesis and fibrogenesis[9] (2). Bile acids downregulate AE2 expression through the formation of reactive oxygen species, with multiple downstream effects including increased expression of the effector cytokines IL-6 and IL-8, and the leukocyte chemoattractant CXCL10[6] (3). Via inhibition of AE2 activity and intracellular accumulation of HCO_3^-, bile acids promote BEC apoptosis and BEC susceptibility to non-bile-acid proapoptotic stimuli[5] (4). Dysregulated over-production of IFNγ in mice recapitulates the features of PBC, resulting in T-cell infiltration of periductular areas, the production of anti-mitochondrial antibody (AMA) in conjunction with B cells and the upregulation of bile acid production (5). The effects are more marked in female mice[3]. BSEP, bile salt export pump; PC, phosphatidylcholine; sAC, soluble adenylyl cyclase.

Further advances in understanding the careful orchestration of liver injury have come from better appreciation of the gut–liver axis, with focus towards the farnesoid X receptor (FXR)–fibroblast growth factor (FGF)-19 signalling pathway. FXR is a central transcriptional sensor of bile acid metabolism and one key target gene in the gut is *FGF19*, which encodes an enterokine released into portal blood following bile acid binding to FXR. Augmentation of this pathway presents opportunities for therapeutic benefit, with the goal of directing enhanced anti-inflammatory, anti-fibrotic and anti-cholestatic mechanisms. In the liver, FGF19 regulates intracellular pathways inhibiting CYP7A1, the rate-limiting enzyme in bile acid synthesis[8]. An FGF19 variant (M70) has been developed as a potential new therapeutic for cholestatic liver diseases, which constitutively inhibits synthesis and hepatic accumulation of bile acids, without

Key advances

- Overexpression of the IFNγ pathway in mice led to an animal model of autoimmune cholangitis that recapitulates the female preponderance of primary biliary cholangitis (PBC)[3]
- Bile-salt-induced apoptosis is regulated by soluble adenylyl cyclase; dysregulation of anion exchanger 2 function sensitizes biliary cells to apoptosis by activating soluble adenylyl cyclase[5]
- Glycochenodeoxycholic acid reduces expression of anion exchanger 2 in biliary epithelial cells, inducing reactive oxygen species and enhancing epithelial cell senescence[6]
- A clinical trial supports the efficacy of obeticholic acid in the treatment of patients failing current care[10], leading to a licensed therapeutic that is predicted to change the clinical course of PBC

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G.M.H. has been on primary biliary cholangitis advisory boards for GlaxoSmithKline, Intercept Pharmaceuticals and Novartis. G.M.H. has been a study investigator for CymaBay Therapeutics, Dr Falk Pharma, FF Pharma, GlaxoSmithKline, Gilead, Intercept Pharmaceuticals, NGM Bio, Novartis and Shire.

carcinogenic potential[8]. In 2016, Zhou *et al.*[9] showed that modulation of bile acid metabolism with FGF19 or M70 in a mouse model of cholangiopathy effectively reversed liver injury, decreased hepatic inflammation and reduced biliary fibrosis. These effects seemed contingent on inhibition of hepatic expression of *CYP7A1* and *CYP27A1*, genes encoding key enzymes in the classic and alternate bile acid synthesis pathways. This approach is now undergoing clinical trials spanning cholestatic liver diseases and NAFLD.

Finally, convincing data have emerged over the past 5 years supporting clinical efficacy for a semisynthetic FXR agonist, obeticholic acid, such that 2016 culminated in a new internationally approved therapy for patients with PBC. In particular, data published in 2016 from a large clinical trial with obeticholic acid[10] added to previous studies showing that FXR agonism has clear, durable effects on relevant markers of active cholestatic liver injury. Obeticholic acid reduces exposure to toxic hydrophobic bile acids by predicted and demonstrated falls in bile acid synthesis through direct and indirect (FGF19) actions on CYP7A1-mediated bile acid synthesis, as well as bile acid excretion by hepatocytes. In conjunction with considerable work on surrogate markers of PBC disease course and outcome, the data from the obeticholic acid trials proved sufficient for product authorisation. Patients with PBC now have access to the first of, hopefully, many new generation treatments for their disease, which have arisen from collective ongoing efforts to finally understand PBC in high-definition.

GUT MICROBIOTA IN 2016

A banner year for gut microbiota research

Wendy S. Garrett

Fascination about the gut microbiota shows no signs of slowing down. The launch of the US National Microbiome Initiative in 2016, and similar efforts across the globe, underscore the continued enthusiasm for microbiome studies in the USA and beyond. Indeed, 2016 has been yet another notable year for gut microbiota research.

Central challenges facing microbiome science are the scale of studies and technological platform development. More specifically, in how large a population can we effectively and efficiently study microbiomes, and how do we develop studies to include the microbiomes of thousands or even millions of individuals that include best technological practices, data reproducibility and open access? Some of these challenges are not intrinsic to microbiome science and are broadly applicable to our culture's increasing thirst and appreciation for big data science. The efforts of the Human Microbiome Project, the MicroBiome Quality Control project, the 'crowd-sourced' American Gut project, the European Union's MyNewGut project and the US National Microbiome Initiative merit praise in their efforts to address these challenges. Herein, key 2016 gut microbiota discoveries are summarized. As is often the case, space constraints and intellectual preferences have both limited and shaped this list of notable papers that aim to fulfil the promise of microbiome science for humankind.

Several independent laboratories and working groups have aimed to define the gut microbiota membership and function of healthy populations. In 2016, one noteworthy paper focused on variation of the faecal microbiome in healthy European populations with extensive metadata. Using the Belgian Flemish Gut Flora Project, the Dutch LifeLines-DEEP study, along with other global data sets, helped identify factors that shape variation in the faecal microbiome and challenged previous assumptions about what factors affect the gut microbiota of adults[1]. A cluster of papers under the aegis of the Human Functional Genomics Group (HFGG)

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explored the dynamic interplay between host genetics, environmental effectors and the microbiota in shaping the human immune response. In Schirmer *et al.*[2], which focused on the gut microbiota and cytokine response, blood and stool samples were collected from 500 individuals of the HFGG cohort (healthy individuals from the Netherlands with extensive metadata). Serum cytokine profiles were generated from both whole blood and participants' peripheral blood mononuclear cells, and stool samples were sequenced for taxonomic and inferred functional profiling. The goal of the study was to link gut microbiome features to interindividual variation in the immune response. The 2016 HFGG studies revealed interesting effects: gender, seasonality and age on monocytic-type cytokine response; specific host genetic loci as key influencing factors on both monocytic-type and T-helper-cell-type cytokine responses; and microbiome inferred functional effects (as opposed to taxonomic effects) on IFNγ and TNF production. The approach and data reported in Schirmer *et al.*[2] serve as an important resource and foundation for future studies on the effect of the microbiome in shaping the human immune response.

Two studies in 2016 from Jeffrey Gordon's laboratory offer hope and true promise for cracking the Gordian Knot of global undernutrition — a problem for which a ready-to-use therapeutic food might not be a sufficiently durable solution. Prior studies of a cohort of Malawian children implicated the microbiome as a principal actor in malnutrition and stunting, and a follow-up study has now identified an association between low levels of human milk oligosaccharide sialylation and severe stunting in Malawian infants[3]. Human milk is not only critical fuel for developing babies but also supports the development of the infant gut microbiota. Using gnotobiotic mice and pigs that consumed a Malawian diet and harboured a group of gut microorganisms isolated from Malawian infants, the group tested the effects of milk oligosaccharide sialylation. In a microbiota-dependent manner, purified sialylated bovine milk oligosaccharides (BMO) in contrast with non-sialylated BMOs, promoted lean body mass gain and altered bone morphology and distinct metabolic patterns in the brain, muscle and liver[3]. In Blanton *et al.*[4], the Gordon group inoculated gnotobiotic mice fed a Malawian diet with gut microbiota from either 6-month-old or 18-month-old healthy and undernourished children. Mice receiving the 'undernourished' microbiota showed impaired growth, and meta –omic profiling identified taxa that correlated with growth and metabolic phenotypes[4]. Two identified species, *Ruminococcus gnavus* and *Clostridium symbiosum,* were sufficient to improve growth and metabolic defects in gnotobiotic mice harbouring the microbiota of donors with malnutrition. These two papers are notable not only for their exemplary approaches to causality testing in microbiome science, but also for their clear translational potential.

The gut–brain axis describes the signalling between the gastrointestinal tract and the nervous system. Although this concept originally linked the enteric nervous system with the central nervous system; for many, the term now includes consideration of gut microbial metabolites affecting emotional, cognitive and other neurological functions. Two 2016 studies rooted in gnotobiotic mouse models provide exciting and compelling mechanistic data regarding the role of the microbiota in neurodevelopment and Parkinson disease.

The role of maternal diet and the outcomes of offspring is a longstanding area of interest. In Buffington *et al.*[5], studies of a maternal high-fat diet (MHFD) in conventional and gnotobiotic mice revealed an intriguing role for diet and gut microbiota in mouse neurodevelopment[5]. The investigators not only observed that MHFD altered the gut microbiota, social behaviour, oxytocin levels and synaptic potentiation in the ventral tegmental area, but they also determined that *Lactobacillus reuteri* was sufficient to correct these MHFD-induced changes and behavioural abnormalities[5].

Mice that overexpress α-synuclein recapitulate several features of human Parkinson disease and generation of gnotobiotic α-synuclein mice enables interrogation of the role of the gut microbiota and their metabolites in motor dysfunction[6]. Interestingly, the mere presence of the gut microbiota enhances the motor deficits in these mice and short-chain fatty acids, via their effects on microglial cells, promoted α-synuclein-mediated neuroinflammation and ensuing motor dysfunction. Collectively, these papers identify key mechanistic roles for selecting microorganisms and microbial metabolites in mice for the study of neurodevelopment processes and neurodegenerative disorders.

Bacterial members of the gut microbiota have garnered enormous attention, but in 2016, studies revealed a role for parasites as members or modifiers of the gut microbiota with potential implications for mucosal immunology and human health. In Howitt *et al.*[7], tuft cells — rare gut chemosensory cells — emerged as key sensors of the mouse symbiotic protozoan *Tritrichomonas muris*. Tuft cells seem to orchestrate the immune response to a variety of pathogenic helminths and employ *bona fide* taste chemosensory pathways to initiate anti-parasite immunity, revealing under-appreciated signalling pathways used to sense gut parasites[7,8]. In Chudnovskiy *et al.*[9], another symbiotic trichomonad was identified, *Tritrichomonas musculis*, which conferred protection from mucosal bacterial infections via

Key advances

- Big data microbiome science is challenging assumptions on what drives human microbiome variation and revealing how the microbiota shape the human immune response[1,2]
- Sialylated milk oligosaccharides promote restoration of a healthy gut microbiota in preclinical undernutrition models[3]
- Select gut microbiota species might be useful partners in restoring growth and metabolic defects in undernutrition[4]
- Gut microbiota and their metabolites promote Parkinson-disease-like deficits in mice[6]
- Symbiotic protozoa and pathogenic helminths shape mucosal immune responses via canonical and noncanonical microbial sensing pathways and affect the gut microbiota[7–10]

host epithelial inflammasome activation and IL-18, but exacerbated susceptibility to colitis and mouse colorectal tumours. As the rates of chronic protozoan colonization and helminth infections have decreased in industrial nations, the rates of inflammatory and allergic diseases have skyrocketed. This observation inspired Ramanan *et al.*[10] to examine helminth infection in mice deficient in the Crohn's disease susceptibility gene *Nod2*. Helminth infection protected these mice from intestinal inflammation and altered the gut microbiota; colonization with inflammatory *Bacteroides* spp. was reduced and a microbiota enriched in *Clostridiales* spp. emerged[10]. The microbiota of individuals from helminth-endemic regions showed similar patterns, which were reversed with anti-helminth therapy. These three studies shed light on different facets of host immune–protozoa–microbiota interactions and support the need for further investigation into their translational applications.

Overall, 2016 was a banner year for gut microbiota science in terms of new functional insights gained from larger cohort studies, novel preclinical models fulfilling the promise of translation of microbiome science, and insights around the biology of parasites, the microbiota and host immunity.

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Competing interests statement
The author declares no competing interests.

FURTHER INFORMATION
American Gut project: http://americangut.org/
Human Microbiome Project: http://hmpdacc.org/
MicroBiome Quality Control project: http://www.mbqc.org/
MyNewGut project: http://www.mynewgut.eu/
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GLOMERULAR DISEASE IN 2016

New advances in the treatment of glomerular disease

Rutger J. Maas and Jack F. Wetzels

Studies published in 2016 provide insights that bring us closer to achieving the goal of personalized therapy for primary glomerular diseases. Moreover, promising renal outcome data with new classes of glucose-lowering agents — SGLT2 inhibitors and GLP-1 agonists — offer new hope for patients with diabetic nephropathy.

As primary glomerular diseases are rare, progress in treatment is slow and randomized controlled trials (RCTs) are limited in number. The discovery of disease-causing antibodies in membranous nephropathy (MN) and mutations in podocyte genes in focal segmental glomerulosclerosis (FSGS), together with the availability of modern immunosuppressive drugs has provided new avenues for individualized therapy, and several important studies were published in 2016. For patients with diabetic nephropathy, the publication of promising renal outcome data with novel glucose-lowering agents might be the beginning of a success story.

The first RCT of rituximab in primary MN — the GEMRITUX study — was published in 2016 (REF. 1). In this open-label trial, patients received maximal conservative therapy with ($n = 37$) or without ($n = 38$) rituximab (375 mg/m^2 on days 1 and 8). The study failed to show a significant difference in the primary end point of complete or partial remission of proteinuria at 6 months (35.1% in the rituximab group versus 21.1% in the control group). Rituximab treatment did, however, significantly increase the rate of decline in anti-PLA2R antibody levels; 50% of anti-PLA2R-positive patients in the rituximab group and 12% of those in the control group showed antibody depletion at 6 months. Most importantly, after 17 months of follow-up, the rate of remission was significantly higher in the rituximab group (64.9%) than in the control group (34.2%). Although these data provide evidence that rituximab can induce proteinuria remission in MN, the fairly high proportion of non-responders in this and other rituximab studies (35–54%) is a cause for concern. Studies with hard renal end points are, therefore, required to evaluate if rituximab reduces progression to end-stage renal disease (ESRD). In addition, the finding of spontaneous remission in 34.2% of patients in the control group underlines the need for better predictors of progressive kidney failure in primary MN to enable avoidance of unnecessary immunosuppressive therapy. A multivariable analysis showed that a high PLA2R antibody titer (>275 U/ml) was associated with a lower likelihood of remission. Moreover, a previous study reported limited efficacy of rituximab among patients in the highest tertile of PLA2R antibody levels[2]. These findings suggest that non-response to rituximab might be the consequence of an inadequately low dose and measurement of anti-PLA2R antibody titres might enable selection of patients who need higher doses to obtain maximal efficacy.

The results of the STOP-IgAN trial question the benefit of immunosuppressive therapy in IgA nephropathy (IgAN)[3]. This open-label RCT included patients with IgAN, relatively stable renal function and persistent proteinuria (>0.75–3.5 g per day) despite 6 months of maximal conservative therapy. 80 patients were randomly assigned to receive supportive care alone (blood pressure target <125/75 mmHg, cholesterol-lowering therapy and lifestyle advice) and 82 patients were assigned to supportive care plus immunosuppression (prednisone in those with estimated glomerular filtration rate (eGFR) >60 ml/min/1.73 m^2 or prednisone plus cyclophosphamide or azathioprine in those with eGFR <60 ml/min/1.73 m^2). Immunosuppressive therapy resulted in higher rates of complete remission of proteinuria (17% versus 5%) and disappearance of haematuria (42% versus 16%), but had no effect on the eGFR end point of a decrease >15 ml/min/1.73 m^2 from baseline at 36 months (22% versus 24%). The findings demonstrate the value of maximal conservative therapy in IgAN. During the run-in phase proteinuria was reduced to <0.75 g per day in about one-third of patients, and during the randomized phase mean blood pressures were consistently <130/80 mmHg and proteinuria was <1 g per day in about three-quarters of patients. The beneficial effects of immunosuppression on proteinuria and haematuria suggest that this therapy reduced glomerular inflammation. Why did this not translate into a beneficial effect on the renal outcome? Clearly, the disease course was very mild in the control group (change in eGFR of 1.6 ml/min/1.73 m^2 per year), thus limiting the power of the study. The data should not, therefore, be used as evidence to withhold immunosuppression in patients with severe IgAN. Although an analysis of the VALIGA study data suggested that corticosteroids are effective in patients with eGFR <50 ml/min/1.73 m^2 (REF. 4), some patients likely require more aggressive therapy.

Steroid resistant nephrotic syndrome (SRNS) with histologic findings of FSGS poses a diagnostic and therapeutic challenge. Many patients respond to ciclosporin and experimental studies suggest that in addition to its

Key advances

- Rituximab can induce remission of proteinuria in patients with primary membranous nephropathy[1]
- Immunosuppression might not improve renal outcomes, but seems to reduce glomerular inflammation in patients with a mild disease course of IgA nephropathy[3]
- Genetic testing might aid treatment decisions in paediatric patients with steroid resistant nephrotic syndrome and/or focal segmental glomerulosclerosis; those with mutations in podocyte genes are unlikely to respond to ciclosporin[7]
- Novel glucose lowering agents — SGLT2 inhibitors and GLP-1 agonists — improve renal outcomes in patients with type 2 diabetes mellitus[8–10]

Table 1 | **Renal outcomes with novel glucose-lowering drugs in patients with type 2 diabetes mellitus**

Study	No. of patients	Composite nephropathy (%)*	Progression to macroalbuminuria (%)	Doubling of serum creatinine (%)	Initiation of RRT (%)	Onset of microalbuminuria (%)
EMPA-REG*[8] *(median follow-up 3.1 years)						
Empagliflozin	4,687	12.7	11.2	1.5	0.3	51.5
Placebo	2,333	18.8	16.2	2.6	0.6	51.2
LEADER*[9] *(median follow-up 3.8 years)						
Liraglutide	4,668	5.7	3.4	1.9	1.2	NA
Placebo	4,672	7.2	4.6	2.1	1.4	NA
SUSTAIN*[10] *(median follow-up 2.1 years)						
Semaglutide	1,648	3.8	2.7	1.1	0.7	NA
Placebo	1,649	6.1	4.9	0.8	0.7	NA

*EMPA-REG: progression to macroalbuminuria (urinary albumin to creatinine ratio >300 mg/g), doubling of serum creatinine level together with eGFR ≤45 ml/min/1.73 m^2 (calculated using the Modification of Diet in Renal Disease formula), initiation of RRT or death from renal disease. LEADER: new onset of macroalbuminuria or doubling of serum creatinine level and eGFR ≤45 ml/min/1.73 m^2, need for continuous RRT, or death from renal disease. SUSTAIN: persistent macroalbuminuria, persistent doubling of serum creatinine level and creatinine clearance <45 ml/min/1.73 m^2 or need for continuous RRT. eGFR, estimated glomerular filtration rate; RRT, renal replacement therapy.

immunosuppressive effect, this agent stabilizes the podocyte cytoskeleton[5]. Children with SRNS have a high frequency of mutations in podocyte genes[6], and a 2016 study reported that treatment response to ciclosporin was dismal in these patients[7]. Among 169 children with SRNS, single-gene causes were detected in 71 (42%) patients. Overall, 79% of mutations were detected in one of three genes: *NPHS1*, *NPHS2* and *WT1*. Only one of 32 patients with a genetic cause of SRNS who received ciclosporin achieved complete remission of proteinuria; five patients experienced partial remission. ESRD developed in 66% of patients with genetic SRNS and was not affected by ciclosporin therapy; patients with partial remission also showed disease progression. By contrast, 79% of patients with non-genetic causes of SRNS responded to ciclosporin, with 60% achieving complete remission. Progression to ESRD was observed in 27% of these patients and was strongly associated with failure to induce remission. This study confirms that SRNS is not a single entity, and that improved diagnostic characterization might assist treatment decisions. The results support routine use of genetic testing in paediatric SRNS, and withholding ciclosporin in those with mutations in *NPHS1*, *NPHS2* or *WT1*. Efficacy of ciclosporin in patients with FSGS owing to other mutations cannot be ruled out and efforts should be made to record treatment outcomes in rare disease registries. How findings in children might translate to adults with SRNS and/or FSGS is unclear.

Diabetic nephropathy is the most common glomerular disease and a leading cause of ESRD. Current treatment for diabetes mellitus is insufficient in preventing diabetic nephropathy and renal outcome trials with agents such as thiazolidinediones, DPP-4 inhibitors, bardoxolone and sulodexide have been unsuccessful. In 2016, renal outcome data with two new classes of glucose-lowering agents were published. The EMPA-REG study showed that the SGLT2 inhibitor empagliflozin significantly attenuated worsening of nephropathy in patients with type 2 diabetes mellitus (T2DM)[8]. Similarly, the LEADER[9] and SUSTAIN[10] trials of GLP-1 agonists (liraglutide and semaglutide, respectively) provided evidence that these agents improve renal outcomes (TABLE 1). None of these studies showed a benefit of the intervention with respect to retinopathy or incident microalbuminuria, but improvements were seen in glycaemic control, body weight and systolic blood pressures.

A reduction in glomerular hyperfiltration is likely responsible for the reduced incidence of new-onset macroalbuminuria with the new agents. SGTL2 inhibitors and GLP-1 agonists might affect glomerular pressure by influencing tubuloglomerular feedback as a consequence of decreased proximal tubular sodium reabsorption. SGLT2 inhibitors and GLP-1 agonists have now been approved for the treatment of T2DM. Their beneficial effects on renal outcome should stimulate their use in patients with class 1–3 chronic kidney disease (they have not been approved for patients with eGFR <30 ml/min/1.73 m^2). Given their different modes of action, future trials should evaluate a combination of SGLT2 inhibitors and GLP-1 agonists. Data from 2016 has thus raised new hope and optimism for patients with diabetic nephropathy.

Overall, 2016 saw important progress in the treatment of glomerular diseases. In the future, measurement of PLA2R antibodies and the development of epitope-specific assays will enable individualized treatment of MN. Children with SRNS and associated FSGS will benefit from rapid genetic testing to inform treatment decisions. Cautious use of immunosuppressive treatment is still warranted in patients with IgAN and the development of predictors of progressive disease will be of major importance. Finally, treatment with SGLT2 inhibitors and GLP-1 agonists will likely improve outcomes in patients with T2DM and combined therapies should be investigated.

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Competing interests statement
The authors declare no competing interests.

GENETICS OF KIDNEY DISEASE IN 2016

Ingenious tactics to unravel complex kidney disease genetics

Kirsten Y. Renkema and Nine V.A.M. Knoers

The genetic background of many kidney diseases is complex and involves multiple genes, genetic variants and molecular pathways. Here, we look at how researchers tackled this challenging topic in 2016, focusing on studies that used ingenious data-integration tactics, which led to new insights into kidney disease aetiology and renal disease progression.

Complex or multifactorial diseases are defined as conditions caused by a combination of genetic and environmental risk factors. To define the contribution of risk factors to the aetiology of a specific trait, it is necessary to investigate well-defined patient cohorts and perform large-scale statistical data analyses. As the genetic background of renal disease and renal disease progression is often complex, the kidney research field appreciates the challenges of defining genetic risk factors. Here, we describe four studies that used ingenious strategies of data integration to identify key genetic determinants of chronic kidney disease (CKD), kidney-disease progression, and focal segmental glomerulosclerosis (FSGS).

> Approaches to identify individuals who are at risk of CKD progression are needed

Pattaro *et al.* aimed to associate genetic variants with estimated glomerular filtration rate (eGFR), a measure of the renal filtration ability used to diagnose and stage CKD[1]. In total, 53 genetic loci were identified as being associated with eGFR, of which 29 loci had already been identified in previous studies. 19 of the 53 loci associated with eGFR among individuals with diabetes specifically. The researchers then combined genome-wide association study (GWAS) meta-analyses, which included data from 133,413 individuals and a replication cohort of 42,166 individuals, with publicly available expression quantitative trait loci (eQTL) and epigenetic data. Pathway enrichment analysis was performed to prioritize genes at the associated eGFR loci[2]. The analyses showed that the genes associated with eGFR are mainly expressed in the kidney and the urinary tract and that they function in pathways important for kidney development, renal transmembrane transporter activity, kidney structure and regulation of glucose metabolism. Integration with epigenetic data demonstrated that the associated variants specifically map to regulatory regions in the kidney. On the basis of these findings, the researchers propose that genetic factors that determine eGFR are largely mediated by direct effects within the kidney, with a possible role for developmental processes in CKD aetiology. The latter finding is consistent with the Barker hypothesis, which states that adult disease can have fetal origins[3].

The strength of this study lies within the combination of three factors. First, meta-analyses on very large amounts of data facilitated the identification of newly associated loci for a complex trait with genome-wide significance. Second, the integration of GWAS data with eQTL and epigenetic data led to further prioritization of the associated loci. Third, by applying molecular pathway analyses, the researchers looked further than the associations identified by GWAS analysis alone and were able to translate their findings into pathways with biological relevance.

Another study looked beyond genetic associations by performing molecular pathway enrichment analyses of single nucleotide polymorphisms (SNPs) associated with CKD progression as identified by GWAS in 1,331 African Americans and 1,476 European Americans from the prospective, observational Chronic Renal Insufficiency Cohort (CRIC) study[4]. The rate of renal function decline varies substantially among individuals with CKD and approaches to identify individuals who are at risk of CKD progression are needed. To better understand the contribution of genetics to CKD progression, a GWAS was performed, separately among African Americans and European Americans and stratified by diabetes status. The researchers identified 12 SNPs associated with eGFR decline in African Americans and six SNPs associated with eGFR decline in European Americans. Four SNPs showed genome-wide significance ($P \leq 5 \times 10^{-8}$) in African Americans. Molecular pathway analyses revealed an over-representation of molecules with renal and urological functions in close proximity to the associated loci. Interestingly, SNPs in a long noncoding RNA (*LINC00923*) associated significantly with non-diabetic CKD in both African Americans and European Americans. Among African Americans, the association of rs653747 in *LINC00923* with CKD progression reached genome-wide significance and could be replicated in a population and phenotype-related cohort. Further investigations should be performed to determine the contribution of *LINC00923* variants to CKD progression. Long noncoding RNAs increasingly form the target of investigation in aetiological studies because they have emerged as an important class of gene-expression regulators[5]. A limitation of this study was that validation of discovery loci was hampered owing to the lack of equivalent cohorts with

Key advances

- Genetic variants associated with chronic kidney disease (CKD) affect molecular pathways relevant for kidney function and kidney development[1].
- Smart data integration methods prioritized molecular key players and pathways involved in kidney disease pathogenesis and CKD progression[1,4,6].
- Genetic variation contributes to the individual differences in CKD progression[4].
- An association was found between a long noncoding RNA (*LINC00923*) and non-diabetic CKD, showing the relevance of noncoding, regulatory regions of the DNA in kidney disease progression[4].
- *WNK1*, *KANK1*, and *ARHGEF17* were identified and validated *in vivo* as susceptibility genes for focal segmental glomerulosclerosis[8].

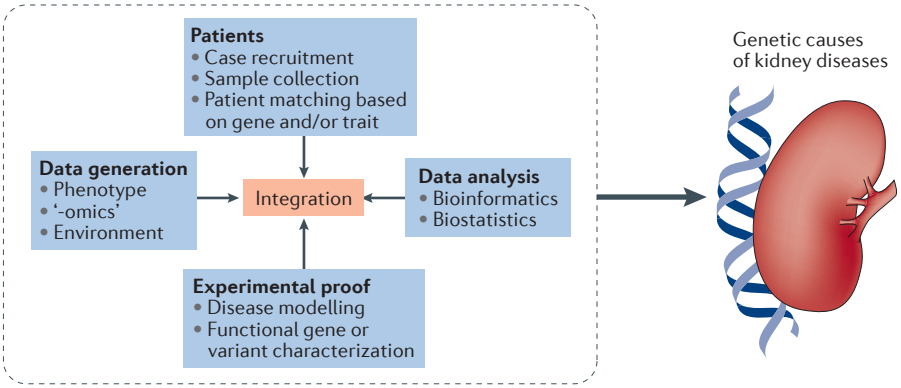

Figure 1 | **Components that are essential for aetiological research strategies for the identification of contributors to complex kidney diseases.** Such studies require consideration of appropriate patient cohorts, approaches to data generation and analysis, and supporting functional experimental data.

longitudinal data, underscoring the importance of large patient registries, extensive data collections and data sharing.

Another key study from 2016 pinpointed genes associated with CKD by combining mouse phenotype information with human GWAS data[6]. The investigators used mouse phenotype data available online from the Mouse Genome Informatics database to prioritize loci associated with CKD. Genes that were prioritized using this approach involved those that, when mutated in mice, resulted in abnormal renal physiology, renal morphology, GFR, or urine protein level. Subsequently,

> Complex disease studies that involve the digestion of large 'omics datasets require ingenious study designs

the matching human gene orthologues were checked for eGFR-associated SNPs as identified by GWAS analysis of individuals in the CKDGen consortium[1]. Significant associations with eGFR in humans were identified for SNPs in two, seven, and 17 genes that caused abnormal GFR, abnormal renal physiology, and abnormal renal morphology in mice, respectively. Furthermore, genes associated with abnormal kidney morphology showed significant enrichment for eGFR-associated SNPs. The integrative approach used by the researchers led to the identification of *CYP26A1*, *BMP4*, and *CYP24A1* as candidate genes for kidney disease pathogenesis, exemplifying how a smart analysis strategy linking GWAS data to biology can be used to investigate the genetics of complex disease[7].

A fourth study explored the genetic background of FSGS[8] — a complex disease in which gene variants can either be sufficient to cause disease or can increase susceptibility to FSGS in combination with environmental factors. Furthermore, FSGS is a rare disease, which precludes the recruitment of large patient cohorts. Instead of using a genome-wide approach, Yu and co-workers applied gene-panel sequencing to search for FSGS-related genes. A selection of 2,500 genes, known to be expressed in podocytes, was sequenced from 214 European American patients. FSGS was confirmed by biopsy in all patients, ensuring a phenotypically homogeneous cohort. Due to the use of a gene panel, fewer statistical tests and comparisons were needed, decreasing the extent of correction for multiple testing. Thus, a focused approach to gene identification, involving a selected region of DNA, has statistical advantages in diseases of limited sample size. Next, potential susceptibility genes were further examined in an *in vivo* high-throughput screening system. The researchers developed a method based on an FSGS mouse model in which genes of interest can be silenced in podocytes specifically. *Wnk4*, *Kank1*, *Kank2*, and *Arhgef17* were validated as FSGS susceptibility genes, as their silencing aggravated proteinuria in the FSGS mouse model.

Complex disease studies that involve the digestion of large '-omics' datasets require ingenious study designs. The four studies on genetic risk factors for complex kidney disease and kidney disease progression discussed here show that the strength of data integration methodologies lies mainly within a combination of using appropriate patient cohorts, informational datasets, functional data, and computational analysis tools[9]. Studies that focus on elucidating the genetic causes of complex diseases require consideration of integrative research strategies (FIG. 1). Of interest, increasing attention is turning to the assessment of variation in the gene regulatory components, such as long noncoding RNAs, to understand the genetic basis of complex kidney diseases. Future large-scale whole genome sequencing and characterization studies will further clarify the effect of noncoding variants on kidney disease aetiology and pathogenesis. The contribution of environmental factors to the aetiology of kidney disease seems underexposed in the field, with the exception of research on the effects of diabetes mellitus. With the current progress in technique developments and bioinformatics opportunities, together with the available collaborative biobank initiatives, the field of complex genetics can and will reveal many more clues on disease aetiology in the near future.

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Competing interests statement
The authors declare no competing interests.

DATABASES
Mouse Genome Informatics: http://www.informatics.jax.org/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

KIDNEY CANCER IN 2016

The evolution of anti-angiogenic therapy for kidney cancer

Chung-Han Lee and Robert J. Motzer

Tyrosine kinase inhibitors that target pro-angiogenic pathways improve progression-free and overall survival in patients with metastatic kidney cancer and were thus tested in the adjuvant setting in studies published this past year. 2016 also saw the emergence of new inhibitors of pro-angiogenic pathways that might represent the next step in kidney cancer therapy.

Kidney cancer has long been understood to be a disease of altered hypoxia signalling. Indeed, loss of Von Hippel–Lindau (VHL) —the most common genetic alteration in clear cell renal cell carcinoma (ccRCC) — leads to the accumulation of hypoxia inducible factor (HIF) transcription factors[1], which activate crucial genes involved in the hypoxic response including those encoding pro-angiogenic growth factors such as vascular endothelial growth factor (VEGF). Targeting the VEGF pathway has therefore been the molecular rationale for the development of multiple agents to treat renal cell carcinoma (RCC), including sorafenib, sunitinib, pazopanib, axitinib, and bevacizumab. In addition, regulatory approval was obtained in 2016 for lenvatinib[2] and cabozantinib[3], which target the VEGF receptor (VEGFR) and putative pro-angiogenic resistance mechanisms mediated by growth factors such as fibroblast growth factor receptor (FGFR) and hepatocyte growth factor receptor (cMET) (FIG. 1). In the metastatic setting, these drugs have demonstrated clear clinical benefits including improvements in progression-free survival and overall survival; however, their efficacy in earlier stages of disease remains unclear and they are therefore being tested as adjuvant therapies after surgery in patients who are at high risk of disease recurrence.

In 2016, two clinical trials — ASSURE[4] and S-TRAC[5] — tested the efficacy of VEGFR-directed tyrosine kinase inhibitors (TKIs) as an adjuvant treatment for RCC. The ASSURE phase III trial[4] randomly assigned 1,943 patients with completely resected high-grade RCC (defined as Fuhrman grade 3 or grade 4 and at least stage pT1b according to the TMN classification system), to receive placebo, sunitinib (50 mg daily for 4 weeks followed by 2 weeks off), or sorafenib (400 mg twice daily). In the original trial design, patients were administered the typical starting dose for metastatic disease; however, the rates of drug discontinuation were high due to toxicity (44% with sunitinib and 45% with sorafenib). After 1,323 patients were enrolled, the clinical trial was amended and starting doses were reduced to 37.5 mg of sunitinib daily for 4 weeks followed by 2 weeks off, or 400 mg of sorafenib daily. With a median follow-up of 5.8 years, the primary end point of median disease-free survival was not significantly different between the groups (79.6 months with placebo, 73.4 months with sorafenib and 70 months with sunitinib; HR 0.97 for sorafenib versus placebo (97.5% CI 0.80–1.17, $P = 0.7184$) and HR 1.02 for sunitinib versus placebo (97.5% CI 0.85–1.23, $P = 0.8038$)). Overall survival at 5 years was also not different between the three groups (80.3% with placebo, 97.5% CI 76.6–84.0%; 80.5% with sorafenib, 97.5% CI 76.8–84.2%; 77.9% with sunitinib, 97.5% CI 74.1–81.9%). This study suggested that in the adjuvant setting, sunitinib and sorafenib were difficult to tolerate and that the use of sunitinib or sorafenib based on an intention-to-treat analysis was not beneficial.

> “the role of TKIs as an adjuvant therapy remains controversial”

The phase III S-TRAC trial[5] randomly assigned 615 patients with completely resected ccRCC, who originally presented with local invasion (tumour stage 3 or greater) or regional lymph node metastasis, to placebo or sunitinib (50 mg daily for 4 weeks on followed by 2 weeks off). Dose reductions and treatment discontinuations in the sunitinib group were 34.3% and 28.1%, respectively, compared to 2% and 5.6% in the placebo group. After a median follow-up of 5.4 years, the primary end point of median disease-free survival on sunitinib was 6.8 years (95% CI 5.8–not reached) compared to 5.6 years (95% CI 3.8–6.6 years) in the placebo group (HR 0.76, 95% CI 0.59–0.98, $P = 0.03$). At the time of data analysis, overall survival was not significantly different between sunitinib (79.1%) and placebo (79.3%; HR 1.01 95% CI 0.72–1.44, $P = 0.94$). These findings suggest an improvement in disease-free survival with the use of sunitinib in the adjuvant setting.

On the basis of these conflicting findings regarding disease-free survival outcomes with sunitinib, the role of TKIs as an adjuvant therapy remains controversial. Whether the discordant outcomes are due to different trial populations or to differences in drug discontinuation rates and dosing, remains speculative. In both trials, median overall survival has not yet been reached, and additional follow-up will be helpful to see if TKIs have an effect on overall survival in the S-TRAC trial. The results of other adjuvant trials with axitinib and pazopanib

Key advances

- The ASSURE trial showed no significant differences in disease-free survival for adjuvant sorafenib or sunitinib treatments[4]
- The S-TRAC trial showed improved disease-free survival with adjuvant sunitinib[5]
- Overall survival data for adjuvant clinical trials remains pending[4,5]
- Novel HIF-directed treatments (PT2399 and PT2385) decreased tumour volume in mouse models[8,9] and stabilized kidney cancer in one patient[10], and are worthy of further investigation

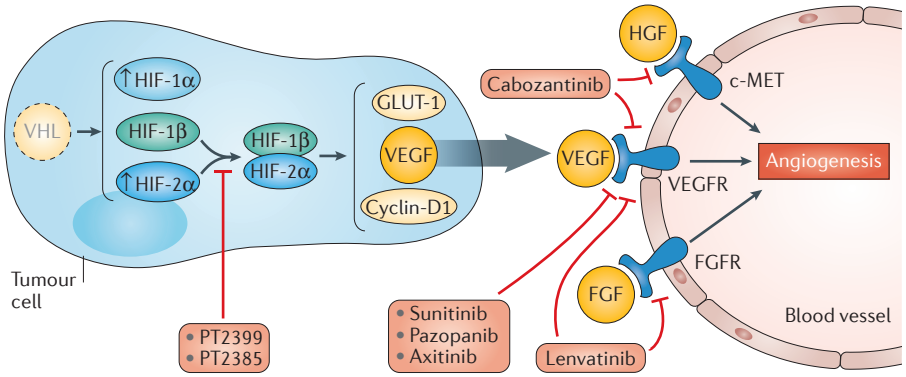

Figure 1 | **Current and future anti-angiogenic therapies.** Loss of *VHL* is a common genetic feature in clear cell renal cell carcinoma that leads to the accumulation of hypoxia inducible factor (HIF)-1α and HIF-2α, and expression of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), G1/S-specific cyclin-D1 and glucose transporter type 1 (GLUT-1). Current anti-angiogenic tyrosine kinase inhibitors (sunitinib, pazopanib, sorafenib, axitinib, bevacizumab) treat kidney cancer by targeting VEGF-dependent pathways at the tumour–endothelium interface. New agents including lenvatinib and cabozantinib also target other tyrosine kinases (VEGFR receptor (VEGFR), hepatocyte growth factor receptor (c-MET), fibroblast growth factor receptor (FGFR)) thought to be critical for resistance to VEGF-directed therapy. A novel class of agents that inhibit HIF-2α-dependent transcription (PT2399 and PT2385) has been developed. These molecules target more directly key pathways in kidney tumorigenesis, downstream of *VHL* loss. HGF, hepatocyte growth factor; FGF, fibroblast growth factor.

(ATLAS[6]; PROTECT[7]) are pending analysis and might shed additional light on the role of TKIs (besides sunitinib) in this setting.

Although strategies that inhibit receptor tyrosine kinases have made substantial advances, direct targeting of HIF has been a long-standing challenge owing to the absence of a known catalytic domain. Loss of VHL can lead to the accumulation of HIF-2α, which dimerizes with aryl hydrocarbon receptor nuclear translocator (ARNT, also known as HIF-1β) and the dimerized complex binds to DNA to induce transcription of HIF-2α target genes. PT2399 is a small molecule that directly binds to HIF-2α and prevents it from dimerizing with ARNT and activating its transcriptional targets8 (FIG. 1). Use of PT2399 led to an >80% reduction in levels of VEGF, G1/S-specific cyclin-D1 (encoded by *CCND1*), and glucose transporter protein type 1 (GLUT-1) in HIF-2α-dependent RCC cell lines[8]. PT2399 also inhibited colony formation in soft agar, a measure of carcinogenic potential, and decreased the volume of tumours induced by orthotopically implanted RCC cell lines[8]. The decreased colony formation and tumour shrinkage seen in response to PT2399 correlated with baseline levels of HIF-2α protein, which suggests that a subset of tumours that depend on HIF2-associated transcription might be particularly sensitive to PT2399.

> “After 11 months of treatment [with PT2385], [a patient with metastatic RCC] remains on treatment and his disease is stable”

The efficacy of PT2399 was compared to that of sunitinib in 22 patient-derived xenografts[9]. This model system recapitulates clinical responses to TKIs such that decreased tumour growth in mice often correlates with tumour shrinkage or disease stabilization in patients. PT2399 decreased tumour growth in xenografts by 60% on average compared to 40% with sunitinib (P <0.0001). Almost half of the xenografts (10 of 22) were sensitive to PT2399 (>80% reduction in tumour volume compared to vehicle control), and these included sarcomatoid and rhabdoid histologic variants and tumours resistant to sunitinib. HIF-2α-dependent transcripts were downregulated in sensitive xenografts, whereas they were unaffected in resistant xenografts (<40% reduction in tumour volume), despite dissociation of the HIF-2α–ARNT complex. Similar to findings in cell lines, the levels of HIF-2α correlated with the response to PT2399. Resistance to PT2399 was acquired with prolonged exposure to the agent, owing to mutations in either HIF-2α or ARNT. A patient from whom a PT2399-sensitive xenograft was generated was enrolled on a phase I trial[10] to assess the efficacy of PT2385, a close analogue of PT2399. This patient had metastatic ccRCC and was heavily pretreated with high doses of IL-2, bevacizumab, sorafenib, everolimus, sunitinib, pazopanib, and axitinib. After 11 months of treatment, he remains on treatment and his disease is stable.

2016 was a year of rapid scientific advancement and improved understanding of the applications of anti-angiogenic therapies. With data from the adjuvant trials, we have begun to explore of the role of systemic TKI treatment for the management of micrometastatic RCC and we await the results of ongoing and future trials to provide further clarity on the efficacy of TKIs as adjuvant treatment. We might also see the development of a novel class of agents that can target the known genetic defects in RCC more specifically than can current treatments. Further studies will be necessary to determine the efficacy and toxicity profile of these agents.

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CRITICAL CARE NEPHROLOGY IN 2016

Managing organ dysfunction in critical care

Ravindra L. Mehta

Approaches to effectively prevent and manage organ dysfunction in critically ill patients remain elusive. Key studies in 2016 highlighted the challenges in finding effective treatments for renal failure in sepsis and assessed the optimal timing of renal replacement therapy initiation in critically ill patients with acute kidney injury.

Management of organ dysfunction in critically ill patients is an area of controversy and active research. The publication of revised definitions for sepsis[1] refocused attention on early interventions with appropriate fluid resuscitation and the use of vasopressors, vasodilators and inotropes to maintain tissue perfusion. Three multicentre trials published in 2016 evaluated approaches to achieve haemodynamic balance without unwanted effects on other organs, including the kidney. In the VANISH trial[2], 421 patients with sepsis requiring vasopressors after fluid resuscitation were randomly assigned in a 2×2 factorial design to one of four treatment groups: patients received infusions of either vasopressin or noradrenaline within 6 h of vasopressor requirement to maintain a target mean arterial pressure (MAP) of 65–75 mmHg; once maximal infusion rates were reached hydrocortisone or placebo were added to the regimen. Patients who were still hypotensive after addition of steroids or placebo also received other open-label catecholamines. The primary outcome of kidney failure-free days did not differ between the treatment groups, nor was any difference found in the secondary outcomes of mortality, length of intensive care unit (ICU) or hospital stay, duration of mechanical ventilation, or serious adverse events. Vasopressin administration was associated with higher urine outputs and a reduced requirement for renal replacement therapy (RRT) than was noradrenaline; however, the need for RRT was left to the discretion of the clinician. Major acute kidney injury (AKI) events occurred in >57% of patients, sustained renal failure at day 28 was seen in >45% of patients, and RRT was initiated in 24% of patients overall.

The Leopards trial[3] tested the efficacy of levosimendan, a calcium-sensitizing inotrope with vasodilating properties, on organ dysfunction in patients with sepsis. Patients who had received fluid resuscitation and vasopressors for ≥4 h to achieve a target MAP of 65–70 mmHg were randomly assigned to receive an intravenous infusion of levosimendan or placebo for 24 h in addition to standard care. Additional inotropes such as dobutamine were added at the physician's discretion to maintain target MAP. No difference in organ failure was observed between the levosimendan and the placebo groups. Secondary outcomes of mortality at 28 days, length of ICU and hospital stay, the proportion of patients with a major acute kidney event over a 28-day period (defined as death, initiation of RRT, or sustained renal failure), and the duration of RRT, were not different between the two groups either, although patients in the levosimendan group had lower MAPs, required higher doses of noradrenaline and were less likely than patients in the placebo group to be weaned off mechanical ventilation.

> "These…trials reflect the ongoing challenges in finding an effective treatment for organ failure in sepsis"

These two trials are complemented by the HYPRESS[4] trial in which 380 patients with sepsis and evidence of organ dysfunction for a duration of <48 h and on vasopressors for ≥4 h were randomly assigned to receive an intravenous bolus of hydrocortisone followed by continuous infusion of hydrocortisone or placebo for 11 days. No difference was observed in the primary outcome of the occurrence of septic shock within 14 days. Mortality at 28 days was relatively low at 8.5% and RRT was required in 12% of patients overall; however, there was no difference between the two groups.

These three trials reflect the ongoing challenges in finding an effective treatment for organ failure in sepsis. Three mechanisms are currently thought to explain sepsis-induced organ failure: impaired tissue-perfusion defects, inflammation and cell hibernation[5]. The three studies presented above suggest that improving tissue perfusion with use of vasopressors or inotropes or by addressing inflammation with steroids is not beneficial. Alternatively, this lack of benefit might relate to a lack of understanding in knowing when each therapeutic intervention is most beneficial. All three studies utilized serum creatinine-based AKIN criteria to characterize renal dysfunction — an approach that might under-estimate the true incidence of AKI, which might be better assessed by urine output criteria. Given that urine volume is a sensitive maker of organ perfusion to guide resuscitation for shock, it is surprising that none of the studies provided any information on this parameter.

Interventions should ideally be implemented when they are most likely to achieve maximal benefit. In the VANISH trial, 21% of

Key advances

- Early use of vasopressin in fluid resuscitated patients with sepsis did not affect the number of kidney failure-free days but did, however, reduce renal replacement therapy (RRT) use compared to noradrenaline[2]
- Infusion of levosimendan for 24 h did not reduce the incidence of severe organ dysfunction or mortality among patients with sepsis compared to standard treatment[3]
- Steroids were ineffective in preventing the development of shock in patients with severe sepsis[4]
- In a single-centre trial, early initiation of RRT in surgical patients with stage 2 acute kidney injury (AKI) was associated with a significant reduction in mortality at 90 days[7]
- In a multicentre trial, delayed initiation of RRT following the onset of complications in patients with stage 3 AKI led to avoidance of RRT in approximately 50%; however, mortality rates tended to be higher among patients who received delayed RRT[8]

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patients already had stage 3 AKI at baseline despite early fluid resuscitation and treatment of systemic inflammatory response syndrome; it is likely that the incidence of stage 1 and 2 AKI was much higher at baseline. Similarly in the Leopards trial, 7% of patients had underlying chronic kidney disease, 29.8% had stage 3 AKI and 17% were on dialysis at time of enrolment. In the HYPRESS trial, >40% had renal dysfunction at baseline even though the study design excluded patients with organ failure of >4 h duration. Since these trials only considered stage 3 AKI or RRT requirement as kidney failure, lower stages of AKI were not accounted for and might have precluded the phases of organ dysfunction where the interventions might have had most benefit.

These studies also illustrate the need for standardized criteria to consider RRT requirement as an outcome measure. Variations in the timing of initiation and discontinuation of RRT are commonplace in critically ill patients and contribute to the lack of demonstrated benefit. The incidence of new RRT in the VANISH and Leopards trials was quite different despite similar enrolment criteria, whereas the incidence of RRT in the HYPRESS trial was lower, perhaps representing the enrolment of patients much earlier in the course of injury.

Two major trials have addressed issues relating to the timing of RRT in the ICU, with opposite results[6]. The single-centre ELAIN trial[7], demonstrated a marked mortality benefit of initiating continuous (C) RRT within 8 h of reaching stage 2 AKI compared to initiating RRT within 12 h of reaching stage 3 AKI (mortality 39.3% versus 54.7%, respectively; $P = 0.03$). By contrast, the multicentre AKIKI study[8] randomly assigned patients in 31 French ICUs to receive either immediate RRT (early strategy) or a delayed strategy in which RRT was initiated if patients developed severe hyperkalaemia, uraemia, metabolic acidosis, pulmonary oedema, or severe oliguria that persisted for >72 h after randomization. More than half the patients were treated with intermittent haemodialysis. The primary outcome of mortality at 60 days was similar in the two groups (48.5% versus 49.7% in the early and delayed strategy group, respectively; $P = 0.79$); however, 49% of patients in the delayed-strategy group never received dialysis. Although dialysis was avoided in almost 50% of patients in the delayed strategy group, those who ultimately required dialysis had worsening metabolic and clinical status on dialysis initiation. Although the differences in mortality were not significant when adjusted for baseline severity of illness,

> “Findings from these studies highlight a need for dynamic risk-stratification tools to identify patients who will or will not need therapeutic intervention”

patients who did not receive RRT had the lowest severity-of-illness scores at baseline and had the lowest mortality (37.1%), followed by patients who received therapy early (48.5%), and then patients who received therapy late (61.8%). The different findings of the ELAIN and AKIKI trials stem from differences in study design and different hypothesis being tested. The ELAIN trial was designed to show a survival benefit for early RRT and included surgical patients with stage 2 AKI, whereas the AKIKI trial was designed to test the hypothesis that delayed RRT would confer a survival benefit in critically ill patients with stage 3 AKI. RRT was consequently initiated at different stages of AKI severity. Moreover, the choice of modality or dose was unrestricted in the AKIKI trial with most patients receiving intermittent haemodialysis, whereas it was limited to CRRT with an effluent volume of 30 (ml/kg)/h in the ELAIN trial.

The ELAIN and AKIKI studies and the design of two ongoing clinical trials (IDEAL-ICU[9] and STAART[10]) suggest that we do not yet have a reproducible approach to evaluate which patients need dialysis in the ICU setting. AKI biomarkers of kidney damage could potentially inform this decision; however, current approaches and study designs do not incorporate an overall assessment of phenotype and do not account for the demand imposed on the kidney by multi-organ failure. I believe that the findings from these studies highlight a need for dynamic risk-stratification tools to identify patients who will or will not need therapeutic intervention, including RRT, for management of their AKI. The timely application of any pharmacological intervention or RRT needs to consider individual patient characteristics, process-of-care elements, and logistics to achieve therapeutic goals. New biomarkers are now available to chart the site and phase of organ dysfunction and should be utilized to create unique biomarker and clinical signatures to define time points for specific interventions. Although the future is bright, we need to know where to look and when to act to make a difference.

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The author declares no competing interests.

RENAL TRANSPLANTATION IN 2016

Novel approaches to improve recipient and allograft outcomes

Paolo Malvezzi and Lionel Rostaing

Kidney transplantation was the focus of numerous publications in 2016. Key studies demonstrated a survival advantage of HLA-incompatible kidney transplantation and suggested that novel approaches such as co-stimulation blockade using belatacept and treatment of antibody-mediated rejection using a C1 esterase inhibitor might prove to be future game changers.

The incidence of chronic kidney disease is increasing worldwide but the availability of deceased kidney donors has reached a plateau. Thus the sole option to enable further increases in kidney transplantation is to increase the number of living donors. In many cases, living donor candidates might be ABO-incompatible and/or HLA-incompatible with their potential recipient. This problem can be circumvented using a kidney paired exchange programme, or if such an approach is not possible or permitted, by performing incompatible kidney transplantation after desensitization therapy[1].

> “progress is being made in safely countering humoral immunity”

In 2016, Orandi *et al.* published a US multicentre study that included 1,025 recipients of HLA-incompatible kidneys from living donors[2]. They found that, despite the need for desensitization therapy, these recipients had significantly better survival at 1 year, 3 years, 5 years and 8 years than did matched waitlisted patients who received kidneys from deceased donors or did not undergo transplantation. This survival benefit was seen even among recipients who had a positive cytotoxic crossmatch result, indicating that progress is being made in safely countering humoral immunity. Although these findings are promising, HLA-incompatible kidney transplantation is unlikely to become a routine procedure because of the frequent complications of acute and chronic antibody-mediated rejection (ABMR), which are difficult and costly to manage.

Various treatment options for acute ABMR are currently being investigated. Recently, Montgomery *et al.* reported data from their placebo-controlled trial of human plasma-derived C1 esterase inhibitor (C1INH) in addition to standard care (plasmapheresis and intravenous immunoglobulin) in 18 patients with acute ABMR[3]. Although the number of participants was small, the results showed a trend towards better graft function and a reduced incidence of transplant glomerulopathy in 6-month protocol biopsy samples in the C1INH group. These encouraging findings are in contrast to disappointing data from several case studies and from the RITUX-ERAH trial, which showed no benefit of rituximab versus placebo in addition to standard care for acute ABMR[4]. Notably, controlled trials in the field of acute ABMR are rare, whereas retrospective case reports are abundant and frequently describe off-label use of powerful, potentially hazardous drugs.

Following kidney transplantation, the main goal is to obtain good long-term (≥20 years) allograft function despite the use of nephrotoxic agents, such as the calcineurin inhibitors (CNIs) ciclosporin and tacrolimus. Although tolerance (that is, normal graft function without immunosuppression), has not yet been achieved, various strategies to reduce or eliminate nephrotoxic agents from immunosuppressive regimens have been tried with conflicting results.

A potential alternative to CNIs in transplant recipients is the fusion protein belatacept, which selectively inhibits T-cell activity by blocking co-stimulation. This agent (at a less intensive dose) was approved by the FDA and EMA in 2011 for the treatment of Epstein Barr virus positive transplant recipients based on 3-year data from the phase III BENEFIT[5] and BENEFIT-Ext trials[6]. In 2016, Vincenti *et al.* reported 7-year follow-up data from the BENEFIT trial, in which 666 *de novo* kidney transplant recipients who had undergone basiliximab induction therapy were randomly assigned to receive either ciclosporin-based immunosuppression or a less intensive or more intensive belatacept regimen, in addition to mycophenolate mofetil (MMF) and steroids[7]. During the first year post-transplantation, the incidence of acute T-cell-mediated rejection was significantly higher in the belatacept groups than in the ciclosporin group, but these events were not associated with worse renal function at 3 years or with the occurrence of *de novo* donor-specific antibodies (DSAs)[5]. Among 447 patients who were followed for up to 7 years post-transplantation, the incidence of death or graft loss at 84 months was significantly higher in the ciclosporin group (21.7%) than in either belatacept group (more intensive 12.7%, less intensive 12.8%; $P = 0.02$)[7]. In addition, mean

Key advances

- Patients who received kidney transplants from HLA-incompatible living donors had superior survival to waitlisted patients who received transplants from deceased donors or did not undergo transplantation[2]
- The addition of a plasma-derived C1 esterase inhibitor to standard care might improve outcomes in kidney transplant recipients with antibody-mediated rejection[3]
- Belatacept-based immunosuppression is superior to ciclosporin-based therapy for long-term preservation of allograft function[7]
- Tacrolimus minimization within the first year after kidney transplantation is not safe and should not be attempted[8]

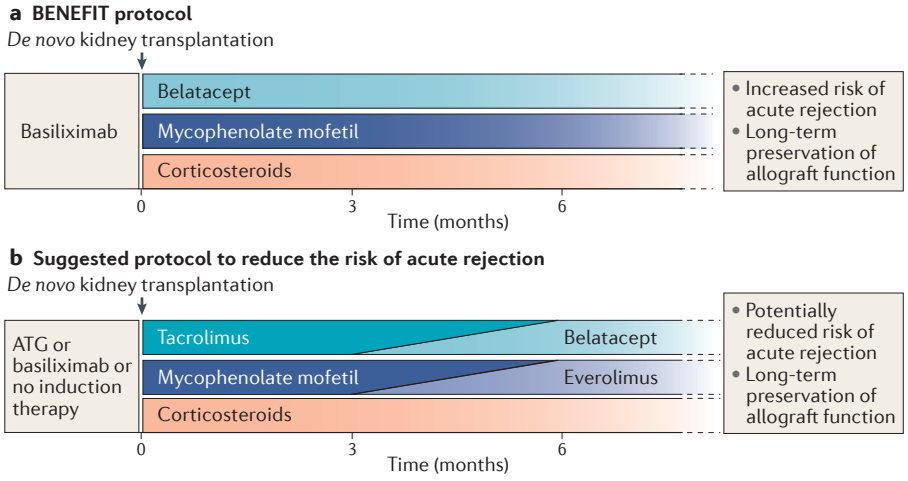

Figure 1 | **Potential strategy for belatacept-based immunosuppression in *de novo* kidney transplant recipients. a** | *De novo* belatacept therapy is associated with an increased incidence of early post-transplantation, low-grade acute rejection. b | An alternative strategy that could potentially avoid early episodes of acute rejection is to use standard tacrolimus-based therapy for the first 3 months post-transplantation and then gradually introduce belatacept and everolimus while weaning off tacrolimus and mycophenolate mofetil, respectively. This strategy is currently being tested in kidney transplant recipients. ATG, rabbit antithymocyte globulin.

estimated glomerular filtration rate (eGFR) increased during 7 years of follow-up in the belatacept groups but declined in the ciclosporin group. At month 84, mean eGFRs were 70.4 ml/min/1.73 m^2, 72.1 ml/min/1.73 m^2 and 44.9 ml/min/1.73 m^2 in the more intensive belatacept, less intensive belatacept and ciclosporin groups, respectively. Moreover, the cumulative rates of development of DSAs at month 84 were significantly lower in the belatacept groups (more intensive 1.9%, less intensive 4.6%) than in the ciclosporin group (17.8%; $P<0.001$). These results demonstrate that belatacept-based immunosuppression is superior to ciclosporin-based therapy for long-term preservation of allograft function.

> “belatacept-based immunosuppression is superior to ciclosporin-based therapy for long-term preservation of allograft function”

The disadvantages of the belatacept regimen are the increased incidence of early post-transplantation, low-grade acute rejection — which could potentially be prevented by using lymphocyte-depleting agents instead of basiliximab as induction therapy — and the need for monthly intravenous infusions of the agent. An alternative strategy to *de novo* use of belatacept would be to use standard tacrolimus-based therapy for the first 3 months after *de novo* kidney transplantation and then introduce belatacept to replace tacrolimus and everolimus to replace MMF (FIG. 1). This approach could potentially avoid early episodes of acute rejection, but enable the long-term benefits of belatacept therapy to be retained. The target population for such a strategy might be recipients of kidneys from living donors and recipients with poor renal function early post-transplantation.

A very recent randomized controlled trial evaluated the feasibility of reducing tacrolimus dose at 4 months post-transplantation in stable, steroid-free kidney transplant recipients[8]. In this study, 186 patients who were receiving extended-release tacrolimus plus mycophenolic acid were randomly assigned to either a 50% reduction in tacrolimus dose (targeted trough concentration >3 μg/l) or to no change in dose (trough concentration 7–12 μg/l). The mean trough levels of tacrolimus were significantly lower in the dose reduction group than the dose maintenance group at 6 months (4.1 ± 2.7 μg/l versus 6.7 ± 3.9 μg/l, $P<0.0001$) and 12 months post-transplantation (5.6 ± 2.0 μg/l versus 7.4 ± 2.1 μg/l, $P<0.0001$). Although eGFR at 12 months post-transplantation was similar in the two groups (56.0 ± 17.5 ml/min/1.73 m^2 versus 56.0 ± 22.1 ml/min/1.73 m^2), the number of rejection episodes (11 versus three, $P=0.016$) and the frequency of subclinical inflammation in surveillance kidney biopsy samples were significantly higher in the dose reduction group. Moreover, donor-specific anti-HLA antibodies appeared in six patients in the dose reduction group but no patients in the dose maintenance group ($P=0.008$). These data demonstrate that tacrolimus minimization within the first year post-transplantation is not safe (trough levels should be maintained at >7 μg/l) and should not be attempted.

Consistent with these findings, a previous trial of tacrolimus withdrawal in kidney transplant recipients with weaning beginning at 6 months post-transplantation was terminated early because of safety concerns[9]. Importantly the data suggested that the number of HLA epitope mismatches, the results of donor-reactive IFNγ ELISPOT assays and the detection of urinary CXCL9 might help to predict which patients would develop acute rejection during tacrolimus weaning[9].

Overall, clinical research in kidney transplantation was very active in 2016. Progress in countering the HLA antibody barrier has enabled excellent survival outcomes with HLA-incompatible transplantation and novel immunosuppressive strategies are starting to emerge.

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Competing interests statement
The authors declare no competing interests.

HYPERTENSION IN 2016

Blood pressure goals, variability and SGLT2 blockade in CKD

Debbie L. Cohen and Raymond R. Townsend

Blood pressure (BP) goals and the management of BP in patients with chronic kidney disease (CKD) remain controversial topics. Key articles in the past year have addressed BP goals in CKD, the use of new agents to slow CKD progression and the effects of visit-to-visit variability in systolic BP on cardiovascular events and renal progression in patients with CKD.

Hypertension contributes to both the progression of chronic kidney disease (CKD) and target organ damage, increasing the risk of cardiovascular disease, stroke and death[1]. Blood pressure (BP) guidelines vary in their recommendations for target BP in patients with CKD with most guideline committees currently recommending a BP goal of <140/90 mmHg for most patients with CKD and some guidelines recommending a lower BP goal of <130/80 mmHg in patients with CKD and proteinuria[2]. The Systolic Blood Pressure Intervention Trial (SPRINT) was a randomized, controlled trial that aimed to identify appropriate systolic (S) BP targets to reduce cardiovascular morbidity and mortality among older individuals without diabetes. 9,361 participants, who were ≥50 years of age with SBP ≥130 mmHg at screening and at risk of cardiovascular disease, were randomly assigned to an intensive SBP target of <120 mmHg or a standard target of <140 mmHg (REFS 3,4). The trial was terminated early, after 3.26 years, when preliminary safety analyses showed a 25% reduction in the primary composite end point of cardiovascular events and a 27% reduction in mortality in participants assigned to intensive SBP control[3,4]. 28% of participants in SPRINT had CKD, defined as an estimated glomerular filtration rate (eGFR) between 20 ml/min/1.73 m^2 and <60 ml/min/1.73 m^2, although patients with polycystic kidney disease, marked proteinuria, treated glomerulonephritis, and renal transplant recipients were excluded. No difference in the pre-specified composite renal end point of a ≥50% decrease in eGFR or development of end-stage renal disease (ESRD) requiring dialysis or transplantation, was observed among participants with baseline CKD; however, the number of ESRD events were small in both groups perhaps due to early termination of the trial and a lower-than-expected decline in eGFR. Acute kidney injury (AKI), defined as a decrease in eGFR of ≥30% to <60 ml/min/1.73 m^2 was more common in the intensive group than in the standard-treatment group (HR 3.49; 95% CI 2.44–5.10; P<0.001), probably due to greater use of antihypertensive therapy in the intensive care group. No evidence of permanent CKD associated with the lower SBP goal exists; however, the possibility of long-term adverse renal outcomes with low SBP targets cannot be excluded. More detailed analyses of patients with CKD in SPRINT are underway to assess the long-term effects of lower BP goals on CKD progression. Currently available data, however, suggest that a lower SBP goal provides cardiovascular protection and reduces mortality in patients with CKD, but does not prevent progression of renal disease. Although the SPRINT study provides important information on managing SBP in non-diabetic patients of advanced age with substantial cardiovascular risk, it is important to remember that these results cannot be generalized to other populations or to all patients with CKD and therefore cannot be used to derive new BP guidelines for patients not included in the SPRINT study population.

When setting a BP goal for a particular hypertensive patient, the decision as to which BP goal to pursue is balanced by treatment risks. The delineation of 'target organ damage' acknowledges that some organs (such as the heart, brain and kidney) are more vulnerable to the effects of high BP than are other organs (such as the liver and spleen). Thus, the chosen BP goal must balance the benefit of target organ preservation against the risks of intervention. A meta-analysis of 44,989 patients enrolled in randomized, controlled trials of intensive versus less-intensive BP-lowering treatment for at least 6 months, evaluated not only conventional primary outcomes such as death, heart attack, heart failure and stroke, but also ESRD and worsening of albuminuria[5]. The investigators included a variety of studies that used different BP goals and achieved differing levels of reduction in BP (from 1.3/0.8 mmHg to as much as 14.2/6.7 mmHg). In addition, some studies included children with CKD and others included patients with diabetes, introducing heterogeneity into the analyses. The meta-analysis showed that intensive BP-lowering treatment was associated with a 14% reduction in major cardiovascular events as compared to the less intensive group. Among the three trials in which worsening albuminuria was included as an end

Key advances

- A lower blood pressure goal provides cardiovascular protection and reduces mortality in patients with chronic kidney disease (CKD), but does not prevent progression of renal disease[4,5]
- Choosing an appropriate blood pressure goal for patients with CKD requires assessment of the benefits of intensive blood pressure control on cardiovascular outcomes and potentially renal outcomes, against the increased risk of adverse effects such as orthostatic hypotension, falls in the elderly and AKI[5].
- The use of empagliflozin when added to standard care (typically an angiotensin-converting-enzyme inhibitor or an angiotensin-receptor blocker) in patients with type 2 diabetes mellitus at high cardiovascular risk is associated with a slower progression of CKD and lower rates of clinically relevant renal events but has no effect on incident albuminuria[7]
- Increased visit-to-visit variability is emerging as a risk factor for death and cardiovascular disease; in patients with CKD, greater visit-to-visit variability in systolic blood pressure predicts death and haemorrhagic stroke, but the underlying mechanisms remain unknown, and no clear interventions are yet available[10]

point (5,224 participants), the meta-analysis identified a significant 10% reduction in worsening albuminuria with intensive BP control compared with less-intensive treatment. In the eight trials that evaluated ESRD as an end point (8,690 participants), however, no significant reduction in the hazard ratio was observed, although a trend for a benefit with more intensive BP control was evident. The lack of effect of tight BP control on the development of ESRD is consistent with the findings from SPRINT. Unlike SPRINT,

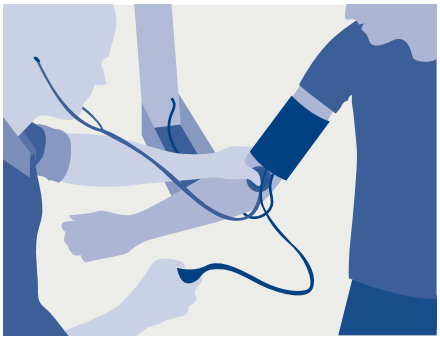
> “These [SPRINT] results cannot be generalized to other populations or to all patients with CKD”

the meta-analysis found an 8% increase in cardiovascular events among patients with CKD who received intensive BP control; however, this effect was not statistically significant. The investigators also noted that the risk of adverse effects, such as hypotension, were up to threefold higher among participants assigned to intensive BP therapy. The value in this study is in its large size, concordance with the SPRINT data in terms of the lack of effect of tight BP control on CKD progression, and the use of ESRD as an end point. The findings from this analysis suggest that future interventions to target hypertension in patients with CKD might require a personalized approach[6].

2016 also saw the first demonstration of renal protection by an agent from a relatively new class of drugs — the sodium–glucose cotransporter 2 (SGLT2) inhibitors. The EMPA-REG OUTCOME trial had previously shown that empagliflozin reduces the risk of major cardiovascular events in patients with type 2 diabetes mellitus (T2DM) and eGFR ≥30 ml/min/1.73 m^2. A secondary analysis to determine the long-term effects of empagliflozin on renal end points showed that fewer patients in the empagliflozin group developed incident or worsening nephropathy (defined as progression to macroalbuminuria, doubling of serum creatinine levels, ESRD, or death from renal disease), than patients in the placebo group (12.7% versus 18.8%; $P<0.001$)[7]. Doubling of serum creatinine levels occurred in 1.5% of the empagliflozin group and 2.6% of the placebo group ($P<0.001$) with a relative risk reduction of 44%. Renal replacement therapy was initiated in 0.3% of participants in the empagliflozin group and 0.6% of those in the placebo group ($P=0.04$), and progression to macroalbuminuria occurred in 41.8% of participants in the empagliflozin group versus 64.9% in the placebo group ($P=0.001$). Incident albuminuria, however, was similar between groups. The incidence of urosepsis was higher in the empagliflozin group whereas the incidence of AKI and hyperkalaemia were lower in the active treatment group. This study showed that empagliflozin, when added to standard care in patients with T2DM at high cardiovascular risk, slowed progression of CKD and led to lower rates of clinically relevant renal events, suggesting this agent might be the first to have a significant role in delaying the progression of renal disease since the introduction of renin–angiotensin system blockers.

That visit-to-visit variability (VVV) in SBP is an independent predictor of death, progression to ESRD, and cardiovascular events, has been reasonably well established in clinical trials of hypertensive patients[8], and in patients who are already on dialysis[9], but data on VVV in patients with CKD who are not enrolled in studies or who are not on dialysis are scarce. A 2016 study leveraged the large captive Kaiser Permanente population of Northern California to address this knowledge gap[10] by evaluating the risks of death, development of ESRD, or cardiovascular events among 114,900 adults with nondialysis CKD over a 3-year period. The researchers observed a significantly higher risk of death (HR 1.22), and haemorrhagic stroke (HR 1.91) among patients in the highest quintile of SBP variability compared with those in the lowest quintile, even after controlling for the mean SBP, the severity of kidney disease, and comorbidities. VVV of SBP did not, however, predict ESRD, heart failure, or ischaemic stroke. Findings from this study showed that a subgroup of individuals at higher risk of death and haemorrhagic stroke can be identified within a broader group of patients with CKD; however, the optimal number of visits and spacing between the visits when screening patients, and most importantly appropriate interventions once candidates are identified are still lacking.

These particular studies are highlighted here because we believe that they showcase heterogeneity in the target organ effects of hypertension and in the degree to which organ damage can be mitigated by BP interventions. The heterogeneity in organ response to BP control might relate to different methodologies used to assess BP. Brachial BP, which is generally obtained in the sitting position and typically used in research studies and in routine clinical encounters, might not reflect



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the actual BP in the brain or kidney, as these organs are upstream and downstream of the measurement point, respectively. Further studies are needed to determine the benefits of reductions in intrarenal pressure on renal outcomes, and whether approaches to decrease intrarenal pressure specifically might reduce the disparity in outcome benefits from BP interventions.

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Competing interests statement
D.L.C. and R.R.T. are SPRINT study investigators.

CNS INFECTIONS IN 2016

2016, the year of Zika virus

Diederik van de Beek and Matthijs C. Brouwer

In 2016, the literature on neurological infections was, understandably, dominated by Zika virus. However, we should not overlook important publications on the treatment of cryptococcal and bacterial meningitis.

Zika virus (ZIKV) sparked global attention in early 2016 after the first cases of brain developmental disorders in newborns were linked to ZIKV infection of their mothers during pregnancy[1,2] (FIG. 1). Initial reports of the *Aedes aegypti* mosquito-transmitted flavivirus epidemic described ZIKV as an exanthemic disease, similar to dengue and chikungunya virus infections[2]. As the epidemic unfolded, however, increasing numbers of newborns with microcephaly were diagnosed, raising suspicions of a causal relationship with ZIKV[2,3].

An autopsy study of a fetus from a ZIKV-infected mother revealed microcephaly with lack of gyration, hydrocephalus, and multifocal calcifications in the cerebral cortex and subcortical white matter, together with mild focal inflammation[1]. Using reverse transcription PCR (RT-PCR) and electron microscopy, Mlakar and colleagues identified ZIKV in the fetal brain tissue in this particular case. A Brazilian study showed a 100-fold increase in microcephaly incidence from November 2015 to February 2016, mirroring the peak incidence of ZIKV infection[3]. After a review of the 1,501 suspected cases, 76 were classified as definite ZIKV-related brain developmental disorders, 54 as highly probable, 181 as moderately probable, and 291 as somewhat probable. Of the affected live-born children, 80% had microcephaly, and the remainder had other congenital structural abnormalities of the brain.

Experimental studies provided further support for a causal link between ZIKV and microcephaly. In neural stem cell, neurosphere and brain organoid models, Garcez and colleagues showed that ZIKV could infect human brain cells and reduce their viability, which might lead to abrogation of neurogenesis during human brain development[4]. The offspring of mice and nonhuman primates infected with ZIKV show similar congenital brain disorders to those found in humans.

ZIKV was also found to be associated with a sharp rise in the incidence of Guillain–Barré syndrome (GBS), an inflammatory demyelinating polyneuropathy (FIG. 1). At the height of the ZIKV epidemic in early 2016, a cluster of GBS cases was observed in Colombia[5]. A total of 401 patients were identified with a neurological syndrome and a history of ZIKV infection, of whom 270 (67%) were diagnosed with GBS. Clinical features were reported for 68 patients, reporting to six university hospitals. The ZIKV infection presented with fever, rash, myalgia, headache, conjunctivitis and arthralgia, after which limb weakness, paraesthesia and facial palsy developed. The mean duration between onset of ZIKV symptoms and GBS was 7 days, and 42% of patients did not have an asymptomatic period between ZIKV and GBS. The latter patients were classified as having a para-infectious onset, whereas those with a symptom-free interval were deemed to have post-infectious GBS[5]. The authors showed that RT-PCR in urine had the highest sensitivity (67%) for demonstrating ZIKV infection in this patient population. Positive serology (IgG or IgM) for ZIKV was identified in 86% of the patients.

The rate of encephalitis among ZIKV infected individuals is low, particularly when compared with other flavivirus infections, such as West Nile and dengue virus. A fatal case of ZIKV encephalitis was reported in Brazil in an otherwise healthy non-pregnant woman[6]. She presented with a rash and arthralgia, after which she rapidly developed leg weakness, dysarthria and confusion. After a gradual deterioration over a period of 10 days, severe brain oedema was observed, which eventually resulted in her death.

2016 was undoubtedly the year of ZIKV, but other important studies on the topic of neurological infections were published. Two large randomized clinical trials evaluated treatment options in HIV-associated cryptococcal meningitis[7,8]. Cryptococcosis is a defining opportunistic infection for AIDS, and is the second most common AIDS-defining illness in Africa. Therapies for cryptococcal meningitis are currently limited to three antifungal drugs (amphotericin B, 5-fluorocytosine and fluconazole) and treatment for complications (in particular, raised cerebrospinal fluid (CSF) pressure), and case fatality rates are high (30%). Oral therapies are desirable for resource-poor settings, which have the highest cryptococcal disease burden.

During brain infections, immune responses can exacerbate cerebral oedema and neurological damage, leading to coma and death. Therefore, adjunctive anti-inflammatory therapies might be of interest in cryptococcal meningitis. A double-blind, randomized, placebo-controlled trial published in 2016 attempted to answer a burning clinical question: what is the role of adjunctive dexamethasone therapy in HIV-associated cryptococcal meningitis? This

Key advances

- Zika virus (ZIKV) infection disrupts neurogenesis in the fetal brain, resulting in neurodevelopmental disorders, most notably microcephaly[3]
- ZIKV infection has also been linked to Guillain–Barré syndrome[5]
- Dexamethasone does not reduce mortality, and increases disability, among patients with HIV-associated cryptococcal meningitis[7]
- Further investigation of adjunctive sertraline in cryptococcal meningitis is warranted[8]
- According to new guidelines, in suspected bacterial meningitis, antibiotic therapy should be started as soon as possible, and no more than 1 h after hospitalization[10]

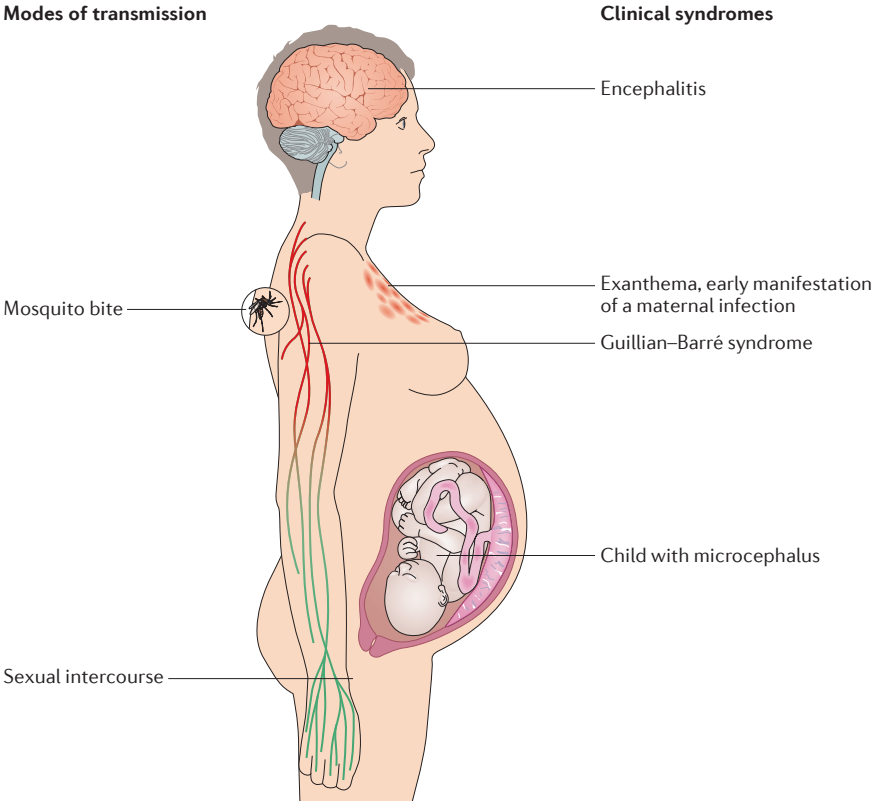

Figure 1 | **Zika virus transmission and clinical syndromes.** Zika virus (ZIKV) is commonly transmitted by the *Aedes aegypti* mosquito, but sexual transmission has also been reported. ZIKV infection presents with exanthema, and might lead to abrogation of neurogenesis during fetal brain development, resulting in microcephaly. During or after ZIKV infection, acute demyelating inflammatory polyneuropathy can develop. In rare cases, ZIKV causes encephalitis.

important trial used a clinical end point, 10-week survival, but was stopped for safety reasons after inclusion of 451 adult patients with HIV-associated cryptococcal meningitis from Vietnam, Thailand, Indonesia, Laos, Uganda, and Malawi[7]. At 10 weeks, mortality rates were similar between groups, but the percentage of patients with disability was higher in the dexamethasone group than in the placebo group (OR 0.42, 95% CI 0.25–0.69, $P < 0.003$). Patients in the dexamethasone group had impaired CSF fungal clearance compared with the placebo group. These findings show that adjunctive dexamethasone has no role in HIV-associated cryptococcal meningitis.

The second trial was an open-label, dose-finding trial on adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis[8]. Sertraline is a selective serotonin reuptake inhibitor with *in vitro* and *in vivo* fungicidal activity against *Cryptococcus* species. 112 participants from Uganda were randomly assigned to three different dose of sertraline for the first 2 weeks, followed by a consolidation dose for 8 weeks. The primary outcome was the 2-week CSF clearance rate for *Cryptococcus*. At 12 weeks, 40% of the study participants had died, indicating the severity of disease. Participants receiving sertraline had faster cryptococcal CSF clearance and a lower incidence of immune reconstitution inflammatory syndrome and relapse than was reported in the past. No clear dose–response relationship between the sertraline dose and early fungicidal activity was found. Nevertheless, further investigation of adjunctive sertraline for cryptococcal meningitis in randomized clinical trials using clinical end points is warranted.

> “ZIKV was … found to be associated with a sharp rise in the incidence of Guillain–Barré syndrome”

The incidence of community-acquired bacterial meningitis decreased following the introduction of conjugate vaccines, but the case fatality rate remains high (17%)[9]. In 2016, European guidelines for the diagnosis and treatment of acute bacterial meningitis were published by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID)[10]. The guidelines state that empirical treatment with dexamethasone should be routine for all adults and children with acute bacterial meningitis in the setting of high-income countries. The guideline strongly recommends starting antibiotic therapy as soon as possible, and no more than 1 h after arrival at hospital[10].

To summarize, 2016 was an exciting year for the CNS infections field. ZIKV infection during pregnancy was found to cause microcephaly and other severe fetal brain defects, and an increased incidence of GBS was reported in areas affected by ZIKV. Randomized clinical trials showed no role for adjunctive dexamethasone and promising effects of adjunctive sertraline in HIV-associated cryptococcal meningitis. Progress has been made in the treatment of bacterial meningitis over the past decade, but we are not there yet, and there is still an urgent need for new adjunctive treatments for cryptococcal and bacterial meningitis.

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Competing interests statement
The authors declare no competing interests.

NEURO-ONCOLOGY IN 2016

Advances in brain tumour classification and therapy

Matthias Preusser and Christine Marosi

Brain tumours encompass a heterogeneous collection of neoplasms, traditionally classified by histopathological criteria. In 2016, the WHO published an updated classification that, for the first time, defines brain tumour types according to integrated histological and molecular parameters. Furthermore, clinical trial results were reported that inform therapeutic decision-making in diffuse gliomas.

May 2016 saw the publication of CNS4+, an update to the WHO classification of tumours of the CNS[1]. For the first time, this new classification integrates histomorphological and molecular parameters for the diagnosis of primary brain tumours. It builds on recent advances, such as the demonstration that different genetic subtypes of morphologically identical tumours have different natural histories, and may differ significantly in their response to treatment.

The new WHO classification is certainly a milestone in the evolution of neuro-oncology, and will change the landscape of brain tumour research by providing a novel reference system for the diagnostic work-up in the clinical setting, as well as for basic and clinical research. It will also introduce new challenges, however. The sophisticated molecular evaluation that is required for a fully integrated diagnosis via the CNS4+ classification is not readily available in every diagnostic laboratory, thereby creating a need to upgrade services or to centralize neuropathology services at national and international levels. Furthermore, clinical trials will need to base patient selection on the new tumour classification, which has potential implications for trial logistics and patient accrual if molecularly defined patient subsets are to be targeted. Hopefully, these efforts will lead to better treatments for well-defined adult and paediatric patient populations.

With regard to the treatment of brain tumours, 2016 brought some important clinical trial results in low-grade gliomas (LGGs, WHO grade II) and anaplastic gliomas (WHO grade III), and in glioblastomas (WHO grade IV) affecting elderly patients (aged ≥65 years). LGGs account for ~15% of all gliomas, mainly affect people in the third and fourth decade of life, are associated with tumour-related epilepsy, have a distinct tendency to recur and progress into malignant gliomas, and limit life expectancy. Treatment strategies for LGGs include neurosurgical resection (which is often not possible due to tumour extent or location), watchful waiting, radiotherapy, and alkylating chemotherapy with procarbazine, vincristine and lomustine (PCV) or temozolomide[2]. The question of which LGG treatment strategy to use in which patient, and how to combine or sequence these treatments, has long been unresolved. Important trial results shedding light on this issue were presented in 2016.

Between 1998 and 2002, Buckner *et al.* recruited 251 patients with LGG who had undergone biopsy or at least partial resection of their tumour[3]. Patients were randomly assigned in a 1:1 ratio to treatment either with fractionated radiotherapy up to 54 Gy with 1.8 Gy per fraction, or to radiotherapy followed by six adjuvant cycles of PCV chemotherapy. After a median follow-up of 11.2 years, it became clear that patients treated with radiation alone had significantly shorter survival than those who subsequently received chemotherapy (7.8 years and 13.3 years, respectively; HR 0.59, $P < 0.003$). Molecular characterization, which was available for 45% of the tumours, indicated greater benefits for patients with 1p/19q codeletions and isocitrate dehydrogenase (*IDH*) mutations.

Another international trial led by the European Organisation of Research and Treatment of Cancer (EORTC) registered 707 patients with histologically proven WHO grade II gliomas, and explored the efficacy of radiotherapy (50.4 Gy, 1.8 Gy per fraction) compared with dose-dense temozolomide chemotherapy (75 mg/m^2 daily for 21 days, in up to 12 cycles of 28 days)[4]. Patients were stratified according to age, WHO performance status, presence or absence of contrast enhancement in MRI, 1p deletion status, and recruitment site. Patients underwent randomization when active treatment was required, and they had to fulfil at least one of the following criteria: age >40 years, new or worsening neurological symptoms, refractory seizures, and radiological tumour progression. The trial included 477 patients, who were followed up for a median of 48 months. Tissue for molecular characterization was available from 318 patients.

> “2016 brought some important clinical trial results in low-grade gliomas … and anaplastic gliomas”

This trial confirmed that patients with *IDH1* mutation and 1p/19q codeletion had the longest periods of progression-free survival (62 months, 95% CI 41 to not reached), followed by the patients showing *IDH* mutations only (48 months, 95% 41–55 months). Patients with wild-type *IDH1* had the shortest period of progression-free survival (20 months, 95% CI 12–26 months). Of note, *IDH*-mutated, non-codeletion patients had a longer period of progression-free survival with radiotherapy than with chemotherapy (55.4 versus 36.0 months, $P = 0.013$). Importantly, temozolomide chemotherapy and radiotherapy did not differ with regard to their adverse effects on health-related quality of life or global cognitive functioning[5].

Key advances

- CNS4+, the updated WHO classification of nervous system tumours, integrates histomorphological and molecular genetic parameters for the diagnosis of primary brain tumours[1]
- Adjuvant chemotherapy with procarbazine, vincristine and lomustine following radiotherapy led to a survival gain in high-risk patients with diffuse gliomas[3]
- The results of the EORTC trial 22033 confirm the predictive value of molecular genetic subgroups in low-grade glioma[4]
- Adjuvant chemotherapy with temozolomide prolongs survival in patients with anaplastic gliomas without 1p/19q codeletion[6]
- Chemoradiation with temozolomide is beneficial for otherwise fit elderly patients with newly diagnosed glioblastoma[8]

For patients with newly diagnosed anaplastic gliomas, van den Bent *et al.* presented the first interim results of the EORTC CATNON trial 26053, which used a 2:2 factorial design to explore the added value of temozolomide as concomitant or adjuvant therapy to radiation (59.4 Gy in 33 fractions)[6]. After a median follow-up of 27 months, the interim analysis showed a hazard ratio reduction of 0.645 (95% CI 0.450–0.926, $P = 0.0014$) for overall survival when adjuvant temozolomide was used. Whether concomitant chemotherapy is also beneficial in these patients will be known in 2021.

For elderly patients with glioblastoma, therapeutic concepts have evolved considerably in recent years, and radiotherapy and chemotherapy monotherapy regimens adapted to patient age and O^6-methylguanine-DNA-methyltransferase (*MGMT*) promoter methylation status have been defined[7]. In 2016, Perry *et al.* reported the results of a Canadian-led phase III trial, in which short-course radiation therapy (40 Gy in 15 fractions over 3 weeks) combined with concurrent and adjuvant temozolomide was compared with radiotherapy alone in fit newly diagnosed glioblastoma patients aged ≥65 years[8]. The addition of temozolomide to radiotherapy extended the median overall survival from 7.6 months to 9.3 months, and quality of life was not diminished with the combined therapy. These findings indicate that multimodal therapy is beneficial and can be considered as a feasible treatment option in fit elderly patients with glioblastoma.

In the field of neuro-oncology, the main advances over the past year lie in a refined classification of primary brain tumours using increasingly sophisticated techniques, and in the extension of our knowledge on indications for and sequencing of classic radiation and alkylating chemotherapy approaches. Unfortunately, like previous years, 2016 failed to deliver positive trial results for biological agents in gliomas. The ACT-IV trial evaluating rindopepimut, a vaccine targeting epidermal growth factor receptor variant III (EGFRvIII), was stopped early for futility, thereby dashing the hopes raised by earlier smaller trials. Hopefully, ongoing trials with other novel agents, such as immune checkpoint inhibitors targeting programmed death 1 (PD-1), or the drug–antibody conjugate ABT-414, which targets amplified EGFR, will produce more-encouraging results in 2017 and beyond[9,10].

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MULTIPLE SCLEROSIS IN 2016

Immune-directed therapies in MS — efficacy and limitations

Bernhard Hemmer and Mark Mühlau

In 2016, new highly active treatment options for relapsing–remitting multiple sclerosis (MS) emerged. At the same time, large clinical trials in progressive MS highlighted the limitations of immune-directed therapies, and called for new strategies to treat disease progression in MS.

During the past two decades, several immune therapies have been established for clinically isolated syndrome (CIS) and relapsing–remitting multiple sclerosis (RRMS). For all the drugs that have been approved to date, efficacy and safety inversely correlate. However, work reported in 2016 has demonstrated that B-cell depletion with CD20-specific monoclonal antibodies (such as rituximab, ocrelizumab and ofatumumab) is a safe and effective treatment strategy for RRMS.

In March 2016, a retrospective study was published in which patients with RRMS were switched from natalizumab to either rituximab or fingolimod[1]. Patients who received rituximab experienced fewer relapses and adverse events than did those who received fingolimod. During the first year, only 2% of patients discontinued rituximab, whereas 24% discontinued fingolimod.

This positive outcome of B-cell depletion was reinforced by data from two identical phase III trials of ocrelizumab in RRMS that were published in December 2016 (REF. 2). In these trials, the efficacy and safety of ocrelizumab and subcutaneous IFNβ1a were compared. Over a period of 2 years, ocrelizumab reduced relapse rates by 46% and 47% (primary end point) in the two trials, and reduced confirmed disability progression by 40% in both trials (secondary end point). Most impressively, ocrelizumab largely abolished inflammatory disease activity as measured with MRI: in the two trials, the numbers of gadolinium-enhancing lesions were 94% and 95% lower in patients treated with ocrelizumab than in those treated with IFNβ. Tolerability and safety of the two drugs was comparable.

The outcomes of these phase III trials and the results emerging from clinical practice suggest that B-cell depletion is a highly effective and safe treatment option for RRMS. However, the long-term effects of B-cell depletion are still uncertain, and caution is warranted in the use of this approach until long-term safety data are available.

A more profound immune therapy for MS that has come to the fore in 2016 is haematopoietic stem cell transplantation (HSCT). Ablation of the adaptive immune system and maturation of a new immune repertoire that lacks autoreactive immune cells as a result of transplantation has the potential to stop autoimmune-mediated damage. In 2016, the results of a phase II trial of HSCT in RRMS and secondary progressive MS (SPMS) were reported[3]. Twenty four patients with highly active disease took part in the trial. One patient died as a result of transplantation-related complications, but 70% of the other patients experienced no disease activity during the 3 years that followed the intervention. The other 30% experienced early and sustained disability progression after transplantation, but without relapses and MRI activity. None of the surviving 23 patients experienced a relapse or developed gadolinium-enhancing lesions for up to 12 years after HSCT.

The results of this study suggest that HSCT is a highly effective treatment for RRMS, with a profound and long-lasting impact on inflammatory disease activity. Although the treatment seemed to fully suppress the development of new lesions, it failed to stop progression in a subgroup of patients. Given the possibility of severe adverse effects and the increasing potency of new immunotherapies, head-to-head trials will be necessary to establish HSCT as a standard therapy in MS.

Further insight into the effectiveness of immunotherapy for progressive MS has been gained from placebo-controlled phase III trials reported in 2016, with relatively sobering outcomes. In particular, the INFORMS trial of fingolimod in patients with primary progressive MS (PPMS) showed that the immunomodulatory drug did not influence 3-month confirmed disease progression (CDP) when compared with a placebo[4], although secondary MRI end points, such as new T2-weighted and gadolinium-enhancing lesions, did indicate suppression of inflammatory activity.

More-encouraging results from the ORATORIO trial were presented in December 2016 (REF. 5). In this trial, ocrelizumab was compared with a placebo in patients with PPMS who were no older than 55 years and had inflammatory cerebrospinal fluid changes. Ocrelizumab reduced confirmed disability progression over 3 months by 24% (primary end point) and over 6 months by 25% (secondary end point), and reduced the change of T2-weighted volume by 90%. The drug was well tolerated, but the observed rate of neoplasms — in particular, breast cancer — was higher among patients who received ocrelizumab than among those who received a placebo (2.3% versus 0.8%). Although ORATORIO was the first trial to demonstrate an influence on disease progression in PPMS, the impact on clinical outcome parameters was still modest.

Key advances

- Research reported in 2016 has demonstrated the efficacy of immune-mediate therapies in relapsing–remitting multiple sclerosis (RRMS) and the limitations of these therapies in progressive MS
- B-cell-depleting therapies have been shown to be highly effective and well tolerated in RRMS[2]
- Autologous haematopoietic stem cell transplantation strongly reduces inflammatory disease activity in active RRMS and secondary progressive MS, but comes with the risk of serious adverse events[3]
- Ocrelizumab is the first drug to reduce disability progression in PPMS, but the impact on clinical outcome parameters is still modest, and the treatment might carry an increased risk of neoplasms[5]
- Patients with primary progressive MS (PPMS) can have cognitive impairments and subclinical MRI activity years before clinical presentation[7,8], suggesting that PPMS has a presymptomatic inflammatory phase

In another phase III trial in SPMS, the monoclonal antibody natalizumab, which is very effective in RRMS, did not show any effect on disease progression[6]. These results indicate that even potent immunotherapies that efficiently target the peripheral immune system and prevent new CNS lesions have — at best — a moderate impact on disease progression, implying that the peripheral immune system no longer predominates in the progressive stage.

If this implication is correct, what are the mechanisms that underlie progression? Two possible explanations have emerged in recent years. First, neuropathological studies have demonstrated the presence of diffuse white matter pathology and cortical lesions associated with meningeal immune infiltrates in progressive MS, indicating the existence of a sequestered immune response in the CNS that is disconnected from the peripheral immune system. The second possibility is that secondary neurodegeneration, triggered by immune-mediated damage to axon–glia structures, develops and causes disease progression. In line with this concept, a careful neuropathological study has demonstrated widespread and pronounced loss of dendritic spines in the cortex in MS that seems to occur independently of cortical demyelination and axon loss[7]. These pathological changes might be a correlate of disease progression that is independent of relapses. The reason for dendrite loss is unclear, but it might be a long-term consequence of widespread low-grade diffuse inflammation in white and grey matter, and in the meninges.

A comprehensive view of progressive MS requires consideration of not only the late and clinically overt progressive phase, but also the probable early events that precede clinical onset of disease. Two important studies published in 2016 have addressed the preclinical phase of PPMS. In one study, Cortese and colleagues linked information in an MS registry to the results of the conscription examination of men in Norway[8]. They found that people who developed RRMS had lower cognitive scores at 2 years before the first demyelinating attack, and those who developed PPMS exhibited cognitive impairment up to 20 years before overt onset of progression. This finding suggests that patients with PPMS can have subclinical disease activity decades before clinical onset.

In the other study, the risk of developing PPMS was investigated in patients with radiologically isolated syndrome (RIS)[9]. Of 453 people with RIS, 128 developed CIS or RRMS, and 15 developed PPMS. People who developed PPMS were a mean of 10 years older and more likely to be men than those who developed CIS or RRMS. The age of onset and the prevalence of PPMS in this cohort were comparable to those in other cohorts of patients with PPMS[10]. Selected patients with RIS who had frequent MRI scans exhibited ongoing asymptomatic MRI activity years before the onset of PPMS.

Taken together, these studies suggest that, at least in a subset of patients, asymptomatic inflammatory CNS lesions occur years before the onset of PPMS. Combined with the fact that only 10% of all new lesions in RRMS are accompanied by clinical symptoms, these findings make it tempting to speculate that PPMS is also preceded by an inflammatory phase, and the lesions remain asymptomatic because they do not affect critical areas, are less destructive, or are few in number. This disease course would place PPMS in the same category as SPMS, with several implications. First, we would need to establish the diagnosis before the onset of progression to enable efficient treatment of PPMS with commonly used immune-directed therapies. Second, for patients in whom we cannot prevent progression (regardless of whether they have PPMS or SPMS), new treatment

strategies are needed that target the putative drivers of progression — compartmentalized immune responses in the CNS, and secondary neurodegeneration. Last, we need to reconsider whether we should separate PPMS and SPMS in future treatment trials that target progressive MS.

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ALZHEIMER DISEASE IN 2016

Putting AD treatments and biomarkers to the test

Eric M. Reiman

Investigational treatments to impede the progression of Alzheimer disease (AD) are being evaluated in clinical trials, and biomarkers to detect and track the disease are being developed and deployed. Recent findings underscore the importance of ongoing clinical trials and biomarker developments in the understanding, treatment and prevention of AD.

Alzheimer disease (AD), the most common form of disabling cognitive impairment in older adults, is among the most daunting public health problems of our time. With more people reaching older age, the urgency to understand, treat and prevent this condition is mounting. Over the past few years, researchers have developed promising biomarker methods to detect and track the disease, long before cognitive impairment manifests. Potential treatments to target elements of the postulated pathogenic cascade have been discovered and are beginning to be tested in clinical trials, incorporating a growing number of biomarkers to help (TABLE 1). Clinical trial findings and biomarker developments reported in 2016 remind us of both the challenges and opportunities ahead.

According to the amyloid hypothesis[1], increased levels of a 42-amino-acid form of the amyloid-β (Aβ) protein in the brain initiate a cascade of deleterious events that lead to progressive symptoms of AD. The postulated cascade begins with aggregation of $A\beta_{42}$ monomers into potentially harmful oligomers and fibrils, the main constituents of Aβ plaques. Inflammatory responses ensue, followed by aggregation, phosphorylation and trans-syn-aptic spread of the intraneuronal protein tau, leading to the formation of paired helical filaments, which are the main components of neurofibrillary tangles. The cascade culminates in dysfunction and degeneration of affected synapses and neurons.

The amyloid hypothesis is espoused by many but not all researchers, and remains the focus of heated debate. The supporting evidence is substantial but largely circumstantial, and clinical trials of anti-Aβ treatments promise to help settle the arguments once and for all. Several of these treatments are now being evaluated in people with cognitive impairment, and even in cognitively unimpaired individuals who, on the basis of

Key advances

- The antibody aducanumab, which binds to and promotes removal of amyloid-β_{42} ($A\beta_{42}$) oligomers and fibrils, dramatically reduced Aβ plaque measurements, and was hinted to slow cognitive decline, in people with mild cognitive impairment or mild AD dementia and a positive Aβ PET scan[4]
- The antibody solanezumab, which binds to and promotes clearance of $A\beta_{42}$ monomers, had little impact on cognitive decline and no impact on PET measurements of Aβ plaque or paired helical filament tau (PHF-tau) deposition in people with mild AD dementia and a positive Aβ PET scan[6]
- ^{18}F-AV1451 PET measurements of paired helical filament tau burden were elevated in the medial temporal cortex and associated with worse memory performance in cognitively unimpaired older adults, were elevated in lateral temporal, parietal and cortical cortex in those with a positive Aβ PET scan, and corresponded roughly to Braak staging of neurofibrillary tangle pathology[7]
- An automated assay dramatically reduced the variability of cerebrospinal fluid (CSF) $A\beta_{42}$ measurements, setting the stage to improve the standardization, precision, power and comparability of CSF biomarkers of AD[8]
- CSF and blood neurofilament light (NfL) chain levels were elevated in mice and humans with Aβ, tau and α-synuclein pathologies, supporting the potential use of blood NfL measurements to help monitor disease progression and treatment effects in protein-based neurodegenerative diseases[9]

Table 1 | **Alzheimer disease processes, biomarkers and therapeutic agents**

Disease process	Biomarkers	Therapeutic agents
Aβ pathology	• Aβ PET* • CSF $A\beta_{42}$ and $A\beta_{40}$* • Others	• Antibodies and vaccines‡ • BACE inhibitors‡ • γ-Secretase modulators • Anti-aggregants
Tau pathology	• Tau PET* • CSF total tau and phospho-tau* • Others	• Antibodies‡ • Anti-aggregants
Neurodegeneration	• Structural MRI* • FDG PET* • Other MRI and PET measures • CSF neurogranin and SNAP-25 • CSF, plasma and serum NfL • Others	• Protective agents • Neurotrophic agents • Bioenergenic agents
Neuroinflammation	• TSPO PET • CSF soluble TREM2 • Others	• Targeted anti-inflammatory agents
Other processes	• Genetic tests* • Other imaging measurements • Other CSF, blood and eye tests • Cognitive and behavioural tests	• Synaptic transmission modulators • Brain stimulation • Diets and lifestyles • Repurposed drugs and supplements • Other agents

*The most extensively used biomarkers. ‡Disease-modifying agents now in clinical trials. Aβ, amyloid-β; BACE, β-site amyloid precursor protein-cleaving enzyme; CSF, cerebrospinal fluid; NfL, neurofilament light chain; SNAP-25, synaptosomal-associated protein 25; TREM2, triggering receptor expressed on myeloid cells 2.

biomarkers or genes, are at increased risk of developing symptoms of AD[2,3].

In 2016, Sevigny *et al.* reported encouraging preliminary findings from a 12-month trial of the antibody treatment aducanumab in people with mild cognitive impairment or mild AD dementia, along with PET evidence of Aβ plaques[4]. This antibody binds selectively to $A\beta_{42}$ oligomers and fibrils, inducing microglia to engulf and remove fibrils. In an initial dose-finding and safety trial, aducanumab dramatically reduced Aβ plaque measurements, and was hinted to slow cognitive decline. Confirmation of the safety data and preliminary cognitive findings for aducanumab in larger and more-definitive trials, which are now underway, would provide conclusive support for the amyloid hypothesis, and would be a game changer in the fight against AD[5].

By contrast, findings from a clinical trial of another anti-Aβ treatment were disappointing. In 2016, Honig *et al.* presented findings from a large and thoughtfully designed 80-week trial of the antibody treatment solanezumab in people with mild AD dementia and PET evidence of Aβ plaques[6]. This antibody binds to and promotes the clearance of Aβ monomers, and is intended to reduce the aggregation of these monomers into potentially damaging oligomers and fibrils. In the trial, solanezumab treatment failed to sufficiently slow cognitive decline, and had no significant impact on PET measurements of Aβ plaque or paired helical filament tau (PHF-tau) deposition. Although trends towards reductions in cognitive and functional decline were observed among the treated patients, the observed effect sizes were small.

There are several possible explanations for the disappointing solanezumab trial findings. For example, the solanezumab dose could have been too low to enable enough antibodies to cross the blood–brain barrier and sufficiently engage their target in the brain, as only one or two in 1,000 antibody molecules cross the blood–brain barrier. Alternatively, the treatment may have been initiated too late in the disease to have a substantial effect. Indeed, the drug may be engaging the wrong Aβ target (monomers) in people with cognitive impairment (when the underlying disease is already extensive), so that pre-existing oligomers and fibrils remain free to exert their potentially harmful effects. Although the possibility remains that critical elements of the amyloid hypothesis are simply wrong, it would be premature to draw that conclusion until different anti-Aβ treatments have failed to work, even in unimpaired people at increased risk, when the disease is less extensive.

AD drug development is not for the faint-hearted, and we need the fortitude to stay the course until the amyloid hypothesis is definitively confirmed or refuted. We need to develop a more diversified portfolio of investigational treatments, clarify the added value of combination therapies, and continue to accelerate the evaluation of prevention therapies. We also need to continue to find new ways to work together and share research data, tools and ideas, and enrolment resources. We need to continue to learn from previous efforts, and conduct trials in the most thoughtful and rigorous way. The increasing availability of AD biomarkers promises to aid this effort.

The best-established AD biomarkers include PET measurements of Aβ plaque burden, PHF-tau burden and cerebral glucose metabolism; MRI measurements of brain atrophy and resting state functional connectivity; and cerebrospinal fluid (CSF) $A\beta_{42}$, $A\beta_{40}$, total tau and phospho-tau levels. These and additional brain imaging, spinal fluid and other biomarker methods continue to be developed, tested and considered for inclusion in observational studies and clinical trials.

In a study reported in 2016, Schöll *et al.* used ^{18}F-AV1451 PET to characterize PHF-tau deposition in cognitively unimpaired older adults[7]. PHF-tau burden in the medial temporal cortex was associated with worse episodic memory performance. PHF-tau in the lateral temporal, parietal and frontal cortex was observed only in unimpaired individuals with PET evidence of substantial Aβ plaque burden, and the regional pattern of PET measurements corresponded roughly with Braak staging of neurofibrillary tangle pathology[7]. Tau PET ligands and image analysis techniques continue to be developed and tested. A need exists to make tau PET ligands more widely available for clinical trials, and to find ligands that are suitable for the investigation of non-AD tauopathies.

A major impediment to the use of CSF biomarkers of AD has been substantial measurement variability among different samples and laboratories. In 2016, Bittner *et al.* demonstrated the ability of an automated Elecsys assay to measure $A\beta_{42}$ levels with much less variability, setting the stage to improve the standardization, precision, power and comparability of $A\beta_{42}$ and other CSF biomarkers in research and clinical settings[8].

CSF neurofilament light chain (NfL) levels have provided an indicator of axonal injury in several neurological disorders. In a study reported in 2016, Basioglu *et al.* found elevated CSF and blood NfL levels in mouse models and humans with Aβ, tau and α-synuclein pathologies[9]. The authors suggest that blood NfL levels could provide an accessible way to monitor disease progression and treatment effects in individuals with protein-based neurodegenerative diseases.

Emerging investigational treatments and biomarker developments (TABLE 1) have the potential to revolutionize the understanding, treatment and prevention of AD. Now is the

time to reaffirm our commitment, put the most promising treatments and biomarkers to the test, and address our ambitious goals.

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MOVEMENT DISORDERS IN 2016

Progress in Parkinson disease and other movement disorders

Joseph Jankovic

In the field of movement disorders, areas that have seen important advances in 2016 include the pathogenesis of Parkinson disease involving extra-CNS α-synuclein pathology, treatment of hyperkinetic disorders with novel dopamine-depleting drugs, and MRI-guided ultrasound surgery for the treatment of essential tremor.

The field of movement disorders — a group of neurological conditions that manifest as hypokinetic or hyperkinetic disorders and other disorders of motor control — includes many areas of immense growing interest. In this update on the most notable advances in this field in 2016, I have chosen to highlight three of these areas: Parkinson disease, hyperkinetic movement disorders and essential tremor.

2017 marks the 200th anniversary of James Parkinson's *Essay on the Shaking Palsy*. Since its publication, much progress has been made in the characterization of the motor and non-motor clinical phenomenology of Parkinson disease (PD), development of effective medical and surgical treatment, and better understanding of possible mechanisms of neurodegeneration that underlie the disease. Nevertheless, many questions remain. In a *Nature* supplement in October 2016 (REF. 1), four big questions were posed: how does PD begin, what is the role of α-synuclein protein, what is the role of the gut in PD, and what is the best way to divide people with the disease into subtypes? Research published in 2016 has addressed some of these questions.

Increasingly appreciated is that nonmotor features of PD contribute to overall disability more than do the typical motor features, particularly in advanced stages of the disease. Consequently, in addition to the traditional subtypes of PD — tremor-dominant PD and postural instability–gait difficulty PD[2] — new subtypes are being recognized, including those based on nonmotor symptoms[3]. Although some clinical and pathological overlap might exist between the subtypes, cognitive and autonomic impairments are more commonly associated with the nonmotor forms of PD, consistent with the presence of more-severe and widespread dopaminergic and nondopaminergic pathology, even outside the CNS.

Three important studies published in 2016 have added to the evidence for nonmotor, extra-CNS manifestations of PD, with important implications. In one study of mice that overexpressed α-synuclein, removing the normal gut microflora — either by raising the mice in a germ-free environment or by treating them with antibiotics — markedly reduced brain α-synuclein pathology, inflammation, and motor deficits[4]. These pathological and behavioural abnormalities worsened, however, when the mice were inoculated with faecal bacteria taken from patients with PD. These findings suggest that gut bacteria influence CNS cellular mechanisms and regulate movement.

This hypothesis is supported by a second study, which showed that the gut microbiota was altered in 34 patients with PD compared with 34 healthy controls[5]. These alterations in the enteric nervous system in patients with PD might contribute to early gastrointestinal dysmotility (for example, delayed gastric emptying and constipation), and might have therapeutic implications — for example, modification of gut microbiota could alleviate symptoms of PD and prevent disease progression.

The third study that was related to extra-CNS manifestations of PD examined the involvement of the skin in PD. This study enrolled 28 patients with PD and 23 healthy controls[6]. Skin biopsy samples were taken from multiple sites, and deposition of pilomotor and sudomotor α-synuclein was quantified. All patients with PD had significantly higher cutaneous α-synuclein deposition than control participants, which distinguished the two groups with a sensitivity and specificity of >90%. If confirmed by other studies, the

Key advances

- Gut microbiota has been shown to differ between patients with Parkinson disease (PD) and healthy controls[4]
- Deposition of α-synuclein in skin biopsy samples in patients with PD suggests that cutaneous deposits could provide a pathological biomarker of the disease[6]
- A novel association between mutation of the *TMEM230* gene and PD suggests that impaired synaptic vesicle trafficking is a mechanism that underlies neurodegeneration[7]
- The approval of the dopamine-depleting drugs deutetrabenazine valbenazine for hyperkinetic movement disorders is expected in 2017 following a positive trial[8]
- Treatment of essential tremor with MRI-guided focused ultrasound thalamotomy has produced encouraging results[10], but longitudinal studies are needed to assess long-term efficacy and safety

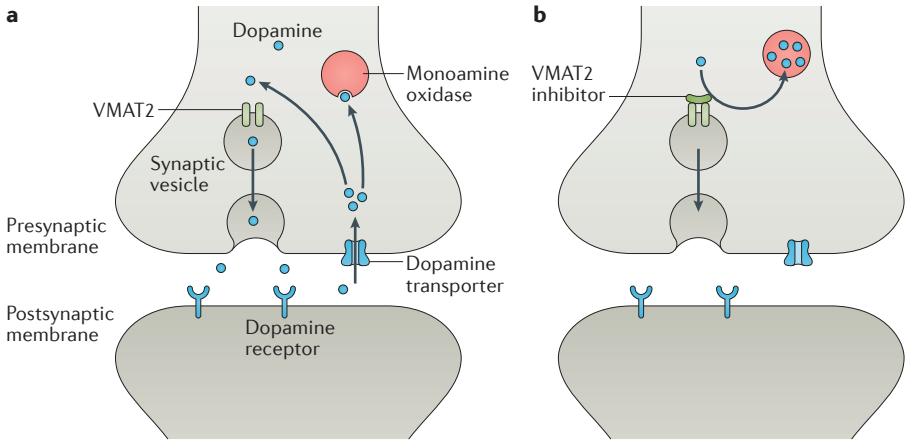

Figure 1 | **Mechanism of action of VMAT2 inhibitors. a** | Normally, vesicular membrane transport type 2 (VMAT2) mediates loading of dopamine into synaptic vesicles for release. Breakdown of dopamine is mediated by monoamine oxidase. **b** | VMAT2 inhibitors block transport of dopamine into synaptic vesicles, reducing dopamine release and depleting dopamine levels through its breakdown by monoamine oxidase.

finding of cutaneous α-synuclein deposition in sympathetic adrenergic nerve fibres that innervate the arrector pili muscles, and in sudomotor (sympathetic cholinergic) nerve fibres, could lead to the development of a diagnostic biomarker of PD.

Converging evidence suggests that α-synuclein pathology starts in the colon and skin before spreading to the olfactory bulb and caudal brainstem, progressing through the brainstem and the diencephalon until it eventually reaches the cortex. The three studies discussed above support this hypothesis, but the concept of prion-like spreading of pathology in patients with neurodegenerative disease has been described as "the most controversial question in the field right now" (REF. 1). The notion that toxic α-synuclein is a major culprit in neurodegeneration is now being translated into early-phase clinical trials of anti-α-synuclein therapeutic strategies.

The 2016 Nobel Prize in Medicine was awarded to Yoshinori Ohsumi for his discoveries of the mechanisms of autophagy, and impairment of autophagy and the ubiquitin–proteasome system continues to be the leading theory for cell death. However, an important new mechanism of neurodegeneration in PD was proposed in 2016. In a family with an autosomal dominant form of PD, Deng *et al.*[7] discovered a novel PD-related mutation in the *TMEM230* gene, which encodes a transmembrane protein in neuronal synaptic vesicles. This finding needs to be replicated in other populations, but we postulate that mutations in the *TMEM230* gene impair synaptic vesicle trafficking and recycling, ultimately resulting in neurodegeneration and a typical PD phenotype.

In the field of hyperkinetic movement disorders, a trial published by the Huntington Study Group in 2016 (REF. 8) is expected to lead to approval of a new dopamine-depleting drug. In contrast to classic neuroleptics that block dopamine receptors, drugs that deplete presynaptic dopamine by inhibiting vesicular monoamine transporter type 2 (VMAT2; FIG. 1) seem to be safer and carry little or no risk of tardive dyskinesia. The VMAT2 inhibitor tetrabenazine is approved by the FDA for treatment of chorea associated with Huntington disease (HD), and is commonly used to treat not only chorea but also other hyperkinetic movement disorders, such as tics and stereotypies. Since the approval of tetrabenazine, other VMAT2 inhibitors, such as deutetrabenazine and valbenazine, have been studied in the treatment of HD-related chorea, tardive dyskinesia and tics associated with Tourette syndrome[9].

In the Huntington Study Group trial[8], 90 patients with HD were randomly allocated to receive deutetrabenazine ($n = 45$) or placebo ($n = 45$). Deutetrabenazine and placebo were titrated to the optimal dosage and maintained for 4 weeks, followed by a 1-week washout period. The difference in maximal chorea scores on the Unified Huntington Disease Rating Scale was 2.5 units in favour of deutetrabenazine; this effect was significant ($P < 0.001$) and was considered to be clinically meaningful. Significant improvements were also seen in secondary end points. The rates of adverse events were similar for deutetrabenazine and placebo. Deutetrabenazine and valbenazine promise to be at least as effective as tetrabenazine, but with a lower risk of adverse effects, such as sedation, insomnia, depression, parkinsonism and akathisia, and an improved pharmacokinetic profile such that administration once or twice daily is possible, instead of three times daily.

Finally, 2016 saw an important development in the treatment of essential tremor with MRI-guided focused ultrasound thalamotomy (FUT). In a multinational trial, 66 patients with essential tremor that persisted despite optimal medical therapy were randomly assigned in a 3:1 ratio to receive FUT or a sham procedure[10]. The patients' tremor was assessed with the 32-point Clinical Rating Scale for Tremor by an independent group of neurologists who were blinded to the treatments. A between-group difference of 8.3 points (95% CI 5.9–10.7, $P < 0.001$) in the mean change from baseline favoured FUT over the sham procedure.

FUT seems to be less invasive than thalamic deep brain stimulation and the results are encouraging, but adverse events were common: 36% of the patients treated with FUT developed gait disturbances, and 36% experienced paraesthesias after the treatment. These adverse effects persisted at 12 months in 9% and 14% of patients, respectively. Long-term studies are needed to confirm the results, although many patients with essential tremor and PD are already requesting this procedure because, in contrast to DBS, it does not involve drilling of burr holes, or placement of intracerebral electrodes or a subcutaneous pulse generator.

These important developments in movement disorders in 2016 highlight several advances, including an improved understanding of the pathogenesis of PD and some novel theories about the role of gut microbiota and the enteric–CNS interaction. New VMAT2 inhibitors will probably be approved in 2017 for the treatment of chorea and tardive dyskinesia, but are also likely to be used for the treatment of other hyperkinetic movement disorders. In addition to immune anti-α-synuclein therapies, immunomodulation of autoimmune movement disorders, such as anti-NMDA receptor encephalitis, is emerging as an important therapeutic strategy.

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STROKE IN 2016

Stroke is treatable, but prevention is the key

Ale Algra and Marieke J. H. Wermer

The past 2 years have seen major breakthroughs in endovascular treatment for acute ischaemic stroke. As highlighted in 2016, we now need to refine the logistics for delivery of this treatment, including patient selection. We should not forget, however, that it is better to prevent strokes in the first place.

In 2015, five ground-breaking trials shook up the stroke community by providing the eagerly awaited evidence for effectiveness of endovascular recanalization therapy in acute ischaemic stroke. In 2016, after the dust had settled, it was time for reflection and refinement.

In the HERMES collective, the investigators of five randomized trials (MR CLEAN, ESCAPE, REVASCAT, SWIFT PRIME and EXTEND-IA) joined forces and performed meta-analyses with individual patient data. The aim was to investigate treatment effects over time and in prespecified subgroups[1,2]. Data from 1,287 patients with proximal anterior artery occlusions were pooled, and the primary outcome was reduced disability on the modified Rankin Scale (mRS) at 90 days.

Overall, endovascular treatment reduced disability at 90 days (common odds ratio (cOR) 2.49, 95% CI 1.76–3.53). The number of patients needed to treat to improve by at least one level on the mRS was only 2.6. A favourable effect was present in all predefined subgroups, including patients older than 80 years of age, those who underwent randomization more than 5 h after symptom onset, and patients not eligible for intravenous alteplase[1]. The odds of better outcomes declined with longer time windows from symptom onset to groin puncture, with cORs of 2.79 (95% CI 1.96–3.98) at 3 h, 1.98 (95% CI 1.98 1.30–3.00) at 6 h, and 1.57 (95% CI 0.86–2.88) at 8 h. The treatment effect remained statistically significant through to 7 h 18 min.

These findings not only show the broad applicability of endovascular treatment, but also emphasize that the 'time is brain' concept holds for thrombectomy. Implementation of thrombectomy will have global implications for the structure of care systems that aim to provide timely treatment to patients with acute ischaemic stroke. One-third of the patients in the trials were transferred from primary stroke centres to advanced intervention centres, highlighting the need to formulate decision rules to predict the presence of large-vessel occlusions, so as to decide which patients to transfer[3]. The symptom-to-groin or door-to-groin time will be a new target to monitor quality of care. Within the chain, all processes in the transport of the patient from home to the angiography suite need to be streamlined. Simple time registration systems based on Bluetooth technology might aid tuning of these multiple steps. Extension of the time window for treatment will increase the number of patients who can be treated and enable implementation of the treatment in rural areas. The HERMES results suggest that treatment up to 8 h after stroke onset is beneficial, but the number of patients in the time window beyond 6 h was too small to draw firm conclusions, hence, further study of the 6–8 h time interval is warranted.

A transient ischaemic attack (TIA) or minor ischaemic stroke is a harbinger of a new vascular — in particular, cerebrovascular — event. This risk is especially high in the initial days to weeks after the event. In a cohort study published in 2016, the TIAregistry.org Investigators enrolled 4,789 patients with a TIA or minor ischaemic stroke[4]. The participants were seen by a stroke specialist at one of 61 sites in 21 countries worldwide. Sites were selected if they had a dedicated system in which patients with a TIA could be seen urgently. The risk of stroke was found to be 2.1% at 1 week, 3.7% at 3 months and 5.1% at 1 year. The 1-year risk of any vascular event (stroke, acute coronary syndrome or vascular death) was slightly higher at 6.2%. A high ABCD2 score, large-artery sclerosis and multiple infarcts at brain imaging each doubled the risk of a new stroke.

The event rates in TIAregistry.org are considerably lower than estimates from studies around the turn of the millennium, which reported a 3-month risk of 12–20% for vascular events. These findings are likely to be explained by earlier and more intensive secondary prevention, including prompt administration of aspirin[5] and the use of statins and antihypertensive medication.

The CHANCE trial, the results of which were published in 2013, indicated that early use of dual antiplatelet therapy prevents stroke recurrence[6]. In this trial, 5,170 Chinese patients with a TIA or minor ischaemic stroke

Key advances

- In pooled analyses of trials on endovascular treatment for acute ischaemic stroke, the number needed to treat to improve by at least one level on the modified Rankin Scale was 2.6 (REFS 1,2)
- These analyses also showed that the beneficial effects of endovascular treatment declined with longer times between stroke onset and groin puncture, but remained statistically significant through to 7 h 18 min (REFS 1,2)
- In patients with a transient ischaemic attack (TIA) or minor ischaemic stroke seen at dedicated TIA clinics, the risk of a new stroke after 3 months was 3.7%[4] — less than half the figure reported 15 years ago
- A substudy of the CHANCE trial showed that the combination of aspirin and clopidogrel was not superior to aspirin alone in carriers of mutations that reduced the ability to metabolize clopidogrel
- Ten modifiable risk factors, which account for >90% of the global burden of stroke, were identified[8]

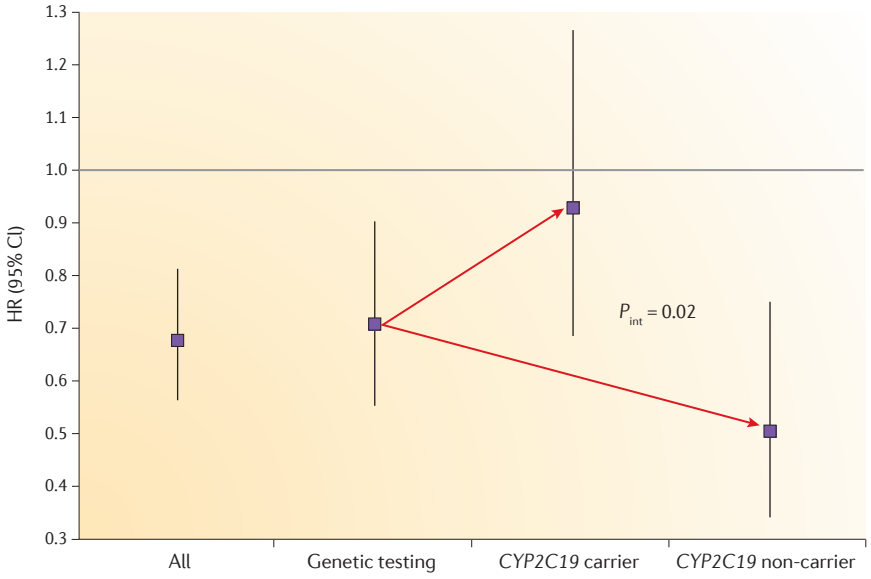

Figure 1 | **The CHANCE trial substudy.** The graph shows the hazard ratios (HRs) and 95% confidence intervals for 90-day stroke recurrence in patients treated with aspirin plus clopidogrel versus aspirin monotherapy. Data are shown for the whole CHANCE cohort[6], the subset of patients who underwent genetic testing for three *CYP2C19* major alleles[7], and the two subgroups who did and did not carry *CYP2C19* mutations that reduced the ability to metabolize clopidogrel. P_{int}, *P*-value for statistical interaction between the two *CYP2C19* carrier subgroups.

were randomly assigned to aspirin plus clopidogrel or aspirin monotherapy. 90-day stroke recurrence was reduced by 32% with the combination therapy. In 2016, the CHANCE investigators reported an important substudy on three *CYP2C19* major alleles that influence the conversion of clopidogrel into its active metabolite in the liver[7]. The genetic analysis was carried out in 2,933 patients, 1,726 (59%) of whom were carriers of *CYP2C19* variants that reduced their ability to metabolize clopidogrel. Among the poor metabolizers, the combination of aspirin and clopidogrel was no longer superior to aspirin (relative risk reduction (RRR) 7%, 95% CI −26% to 31%), whereas in patients who metabolized clopidogrel efficiently, combination therapy was even more effective than in the overall trial (RRR 49%, 95% CI 25–65%). A statistically significant interaction of the treatment effect between the two subgroups ($P=0.02$) was observed (FIG. 1).

Of note, the CHANCE findings pertain to the Chinese population, in which the prevalence of *CYP2C19* variants affecting clopidogrel metabolism is around 60% — much higher than in European populations, for example, where the prevalence is about 30%. However, if these findings are replicated in non-Chinese populations, they could have important implications for the acute management of patients with a TIA, and genetic testing might become a part of the armamentarium of the emergency department.

Stroke is a global problem, affecting 17 million people each year. The advent of high-tech personalized approaches carries the risk of further increasing the disparities in stroke care between high-income and middle-to-low-income countries. Stroke mortality and disability have decreased over the past decade in high-income countries, but stroke is still the second most common cause of death in low-income countries. The majority of people around the world have no access to intravenous alteplase, thrombectomy, stroke unit care or 24 h TIA services, so we should place more emphasis on stroke prevention.

In a mega-case–control study of 13,447 patients with a first acute stroke (10,388 with ischaemic stroke and 3,059 with intracerebral haemorrhage) and 13,472 controls, the INTERSTROKE investigators sought to identify potentially modifiable stroke risk factors[8]. Ten such factors were found: hypertension, smoking, diabetes mellitus, physical activity, diet, psychosocial factors, abdominal obesity, alcohol, cardiac causes, and apolipoproteins. These ten risk factors together account for over 90% of the population attributable risk, and were found to apply to all major regions of the world, as well as all ethnic groups, sexes and ages.

These findings were in line with the results of a subanalysis of the Global Burden of Disease study, which focused on stroke in 188 high-income, middle-income and low-income countries from 1990–2013 (REF. 9). In this subanalysis, the population attributable fractions of stroke-related disability-adjusted life years (DALYs) were calculated in relation to potentially modifiable environmental, occupational, behavioural, physiological and metabolic risk factors, and in different age and sex groups. Again, over 90% of the global stroke burden was ascribed to modifiable risk factors. Importantly, almost one-third of the worldwide burden of stroke was attributed to air pollution.

The year 2016 showed us the promising future of high-tech and personalized stroke care, but also taught us not to forget the need for primary stroke prevention. Investments in global prevention programmes are urgently needed to substantially improve health around the globe. To this end, the World Stroke Organisation campaigns with the slogan "stroke is treatable; lives can improve with better awareness, access and action" (REF. 10).

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Competing interests statement
The authors declare no competing interests.

REGENERATIVE MEDICINE IN 2016

Important milestones on the way to clinical translation

Daniel A. Grande

Regenerative medicine can be viewed as 'tissue engineering V2.0'. Discoveries and novel applications of technology advanced the field considerably in 2016, with the use of new biomaterials, stem cells and biologically active molecules.

This year saw strides forward in all three arms of the tissue engineering triad — cells, scaffolds and signalling molecules. Developments in 2016 included the identification of a new stem cell population in fibrocartilage and its signalling pathway, a refined method for 3D bioprinting of collagenous structures with micro-heterogenous domains, and whole-joint tissue resurfacing technology with tunable properties to thwart the effects of an inflammatory environment.

There is no doubt that the field of regenerative medicine has enthusiastically embraced the paradigm-shifting technology of 3D bioprinting. Although there have been important developments in printing geometrically complex shapes in the past 3 years, the ability to reproduce the heterogeneous spatial arrangement of biologically complex structures has eluded investigators to date. In 2016, Rhee and co-workers demonstrated a bioprinting technique to generate constructs with discrete microdomains that exhibit distinct material properties[1]. The authors developed a novel 'bioink' with a high-density collagen biogel (10-fold higher concentration of collagen compared with previously studied hydrogels[2]) and used a modified build plate that was heated to 37 °C to enable rapid polymerization of the hydrogel, resulting in improved fidelity of the printing process. This novel bioink preparation displayed excellent cell viability with cells starting to align along the collagen fibres in an organized fashion via integrin receptors[1]. Using this methodology, cells and collagen fibres can be oriented in specific directions to resist the tensile and circumferential forces found in the meniscus. This technology marks a step closer to biomimetic replication of the micro-cytoarchitecture of complex collagen structures.

Importantly, the mechanical properties of the bioink produced by Rhee *et al.*[1] are superior to those of other hydrogel bioinks currently available for printing soft tissues such as cartilage[3], thus marking progress towards the development of constructs capable of load-bearing. This report signals the first demonstration of successful bioprinting of constructs that replicate the complex microarchitecture of the knee meniscus, which has organized collagen bundles in load-oriented geometries. Ultimately, heterogeneous 3D-printed constructs could have applications in a number of other musculoskeletal tissues such as articular cartilage, which contains discrete layered zones with differing material properties and Benninghoff arcades (the unique cytoarchitecture of oriented collagen).

Tissue engineering strategies have traditionally relied on the use of cells, whether fully differentiated or stem cells, that are expanded in culture and then used alone, seeded onto porous scaffolds or delivered by hydrogel. An alternative strategy is to recruit endogenous stem cells, which obviates the need for stem cell isolation, culture and delivery. A 2016 article by Embree and co-workers[4] details the discovery of a new population of fibrocartilage stem cells (FCSCs) located in the superficial zone of the condyles of the temporomandibular joint (TMJ). The authors convincingly demonstrated the generation of cartilage, bone and haematopoietic marrow from a single FCSC. This finding suggests the potential use of culture-expanded FCSCs for cell-based therapies for fibrocartilage structures. Embree *et al.*[4] further showed that the maintenance and phenotypic status of FCSCs is regulated through the canonical Wnt signalling pathway, and that the Wnt signalling inhibitor sclerostin maintain the FCSC population and joint homeostasis. They demonstrated in another experiment the ability of FCSCs to be chondroprotective in a rabbit model of TMJ degeneration[4].

Fibrocartilage structures in the body include not only the TMJ but also the meniscus, vertebral discs, and tendon-to-bone enthesis; options for the treatment of injury to these structures are limited. The study by Embree *et al.*[4] suggests sclerostin might have use as a potential therapeutic in fibrocartilage degeneration through its effects on resident FCSCs. Whether the resident population of stem cells in the superficial zone of hyaline cartilage, which makes up other joints such as the knee and hip, will respond to a therapeutic such as sclerostin remains to be seen[5]. If so, this approach would be broadly applicable to degenerative joint diseases such as osteoarthritis.

The recruitment of endogenous stem cells is an attractive strategy for the treatment of cartilage injuries and the characterization of FCSCs by Embree and colleagues[4] is an important next step in developing this

Key advances

- 3D bioprinting can produce biomimetic constructs that replicate the complex microarchitecture of the knee meniscus[1]
- A resident population of fibrocartilage stem cells could be exploited for cartilage regeneration and repair via manipulation of the Wnt signaling pathway[4]
- The combination of gene therapy and tissue engineering can produce both cell-based and acellular engineered constructs with tunable and inducible anticytokine effects[9]

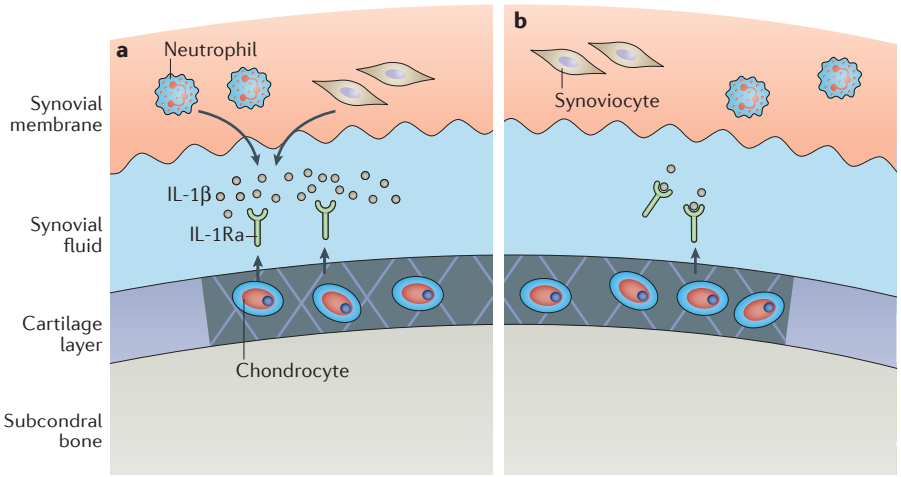

Figure 1 | **Gene therapy combined with tissue engineering produces engineered constructs with therapeutic anticytokine effects. a** | Chondrocytes or chondro-differentiated adipose-derived stem cells are genetically modified to express IL-1 receptor antagonist (IL-1Ra) when treated with doxycycline. **b** | IL-1β level in the synovial fluid is reduced after induction of IL-1Ra release.

treatment strategy. This group had previously demonstrated the recruitment of endogenous stem cells using an anatomically shaped scaffold loaded with transforming growth factor (TGF) β3 to induce chondrogenesis and chemoattract mesenchymal stem cells (MSCs), resulting in hyaline cartilage resurfacing[6]. The new data published in 2016 suggest a similar population of FCSCs might exist in the meniscus[4], which is one of the more difficult structures to repair with orthopaedic surgery.

The applications for tissue engineering technologies have now progressed beyond the treatment of focal cartilage defects to that of entire joint surfaces using novel woven scaffolds seeded with cells (either differentiated chondrocytes or stem cells). In almost all cartilage repair studies to date, however, little attention has been given to the environment into which an anabolic treatment to restore cartilage is placed. Following damage to the joint there is an increase in inflammatory mediators such as IL-1β and TNF, which could compromise the integrity of a tissue-engineered construct as well as the rest of the joint surface. Additionally, IL-1β, which is a key player in the aetiology and progression of OA, has been shown to inhibit MSC-based repair of articular cartilage[7,8]. Gene therapy using IL-1 receptor antagonist (IL-1Ra) is promising as a potential therapeutic to counter the effect of IL-1β. In 2016, Moutos *et al.*[9] combined IL-1Ra gene therapy with tissue engineering to produce engineered constructs with therapeutic anticytokine effects (FIG. 1).

Moutos and co-workers expanded upon their initial work in developing anatomically designed whole-joint resurfacing techniques[10] by adding the capability to provide anti-inflammatory properties, thus protecting the engineered construct in an inflammatory environment. An advanced textile manufacturing approach was used to produce orthogonally oriented fibres that provide mechanical functionality immediately upon implantation with patient-specific geometries; the gene therapy approach used a scaffold-mediated lentivirus transduction technique to enable controlled delivery of anticytokine therapy[9]. The study characterized the ability of the IL-Ra-expressing constructs to inhibit the effects of IL-1β on the development of cartilage *in vitro*.

Such a scaffold-mediated lentivirus transduction technique could be used as an acellular approach utilizing endogenous stem cells in other joint-resurfacing applications. In addition, this approach could be adapted for other growth factors, such as TGFβ, which has been shown to be an important morphogen in the recruitment of stem cells and their differentiation toward cartilage in other acellular models of scaffold-based cartilage repair[6]. This method of transduction results in high titres of anticytokine therapy in an exogenously tunable and inducible manner. The combination of textile manufacturing and scaffold-mediated lentiviral transduction provides a tissue engineering strategy for total-joint resurfacing. The ability of the gene construct to self-regulate the production of IL-1Ra in response to variations in IL-1β concentration within the synovial environment — that is, acting as a feedback mechanism — as well as the use of multiple morphogens in combination to simultaneously provide vigorous support to the cartilage layer would be substantial enhancements of this system. Key challenges to the clinical translation of this technology include determining how well this resurfacing approach will perform *in vivo*, whether this construct will integrate with the subchondral bone to form a solid base for joint motion, and how complex such a surgery will be to perform.

Progress made in 2016 moves the field of regenerative medicine closer to clinical translation. Improved resolution in 3D bioprinting foretells the use of off-the-shelf meniscus, obviating the need for allografts. Harnessing the power of FCSCs, for use as allogeneic cell-based therapies or by exploiting the Wnt pathway with sclerostin or a small-molecule analogue, could present promising therapies for difficult-to-treat fibrocartilage injury. Finally, the concept of providing a whole-joint tissue-engineering solution, rather than treating only cartilage injury, reaches a new level of sophistication with constructs capable of thwarting the effects of an inflammatory environment. Challenges remain but can be met by careful and continued refinement of these developments.

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Competing interests statement
The author declares no competing interests.

SYSTEMIC LUPUS ERYTHEMATOSUS IN 2016

Gene expression profiling comes closer to the clinic

Guillermo Barturen and Marta E. Alarcón-Riquelme

Gene expression profiling has been used for the first time to stratify patients with systemic lupus erythematosus (SLE) into potentially useful clinical groups, and also to further understand differences in the cell-specificity and nature of the interferon signature typical of SLE and other autoimmune diseases.

Genome-wide gene expression analyses and other so-called 'omics' analyses are providing a wealth of information that is helping to grow our understanding of the mechanisms of systemic lupus erythematosus (SLE). However, the most important feature of these 'omics' analyses is their usefulness in the clinical setting, in particular for the stratification of patients into clinical groups that might be amenable to personalized therapies and for future studies on the pathophysiology of the disease.

We believe that three papers in particular from 2016 have provided new and important information on potential mechanisms behind the type I interferon (IFN) signature in SLE (FIG 1). Two studies investigated differences in the expression of the type I IFN signature across diseases and cell types[1,2]. The third study went further; based on modular analysis, the authors stratified patients with SLE according to relevant biological features involved in disease activity patterns[3]. This study represents the first attempt to uncover the real complexity of SLE beyond the type I IFN signature.

De Jong *et al.*[1] analysed the type I IFN signature in several diseases, including SLE, rheumatoid arthritis (RA), inflammatory idiopathic myopathies and multiple sclerosis. Type I IFN responses were analysed according to whether they were IFNα-related or IFNβ-related and by the degree of involvement of each type I IFN. The authors used blood from patients with multiple sclerosis receiving IFNβ therapy as the reference for the IFNβ signature and blood from patients with SLE for the IFNα response. Patients from both reference groups who had a positive type I IFN signature were then selected (patients expressed an average of 23 interferon response genes, which is 2.5–4 times higher than the average for healthy individuals), and unsupervised clustering was performed, followed by differential expression analysis. In this way, the authors could differentiate between the groups of patients and were able to define IFNα and IFNβ signatures in these groups. Two scores were constructed based on the average expression of IFNα and IFNβ signalling genes: GC-A (SLE–IFNα) and GC-B (multiple sclerosis–IFNβ), and a GC-A:GC-B log ratio was used in the analysis of the remaining diseases. Interestingly, in untreated patients with multiple sclerosis and inflammatory idiopathic myopathies, a dominance of the GC-A score was observed (although there was important heterogeneity in patients with multiple sclerosis, some of whom also had a GC-B score), whereas in patients with RA, both scores were equally involved. The authors have previously identified a baseline type I IFN signature that is related to unresponsiveness to IFNβ treatment in patients with multiple sclerosis[4], and a similar phenomenon has been observed in patients with RA who are unresponsive to anti-TNF treatment[5]. This study attempts for the first time to dissect the type I IFN signature within and across diseases, providing some hints regarding the pathophysiology of the type I IFN signature itself, and suggesting the existence of not just one, but at least two type I IFN signatures. The authors' suggestion of differential signalling in individuals having the GC-A versus the GC-B cluster seems reasonable, and might shed light on the mechanisms of initiation of such responses if they could be linked to specific types of initiating viral infections or other intracellular inducers. From this work it is clear that SLE is an IFNα and not an IFNβ disease.

Flint *et al.*[2] also investigated the type I IFN signature, primarily looking for differences in the type of signature expressed by various cell types (neutrophils, $CD4^+$ T cells, $CD8^+$ T cells and monocytes) across four different immune-mediated conditions, including SLE, and healthy individuals. These authors used weighted gene expression network analysis (WGCNA), which identifies gene modules in the data based on co-expression (a method widely used after the publication of Chaussabel *et al.* in 2008 (REF. 6)). Once modules were obtained, one of them was selected for each cellular population as the most representative type I IFN signature based on gene composition, correlation with SLE diagnosis and correlation with a 21-gene core type I IFN signature expression profile. An extensive analysis of the 1,288 genes within the selected modules (1,150 unique to myeloid subsets and only 11 unique to T cells) was then performed to compare between diseases and cellular populations. Examining the median expression of the type I IFN core genes (67 genes shared by the four modules), most of them were found to be highly expressed in myeloid cells and neutrophils, whereas only a few of them had increased expression in T cells. However, high expression of the T-cell-specific modules seemed to be an exclusive feature of patients with SLE, unlike monocyte-specific and neutrophil-specific modules, which presented similar expression levels across other diseases and controls. As the authors discuss, the similar neutrophil type I IFN signature across conditions seems to be concordant with the importance of basal type I IFN signalling in maintaining myeloid populations, whereas it does not seem to be necessary in T cells. On the other hand, the specific expression of T-cell modules in patients with SLE (in agreement with findings

Key advances

- SLE is predominantly an IFNα and not an IFNβ disease[1]
- The type I IFN signature carried in patients with SLE is highly T-cell specific and unique to the disease[2]
- Patients with SLE can be stratified into seven groups, with signatures (representing different cellular mechanisms) that could help to differentiate patients in future clinical trials[3]

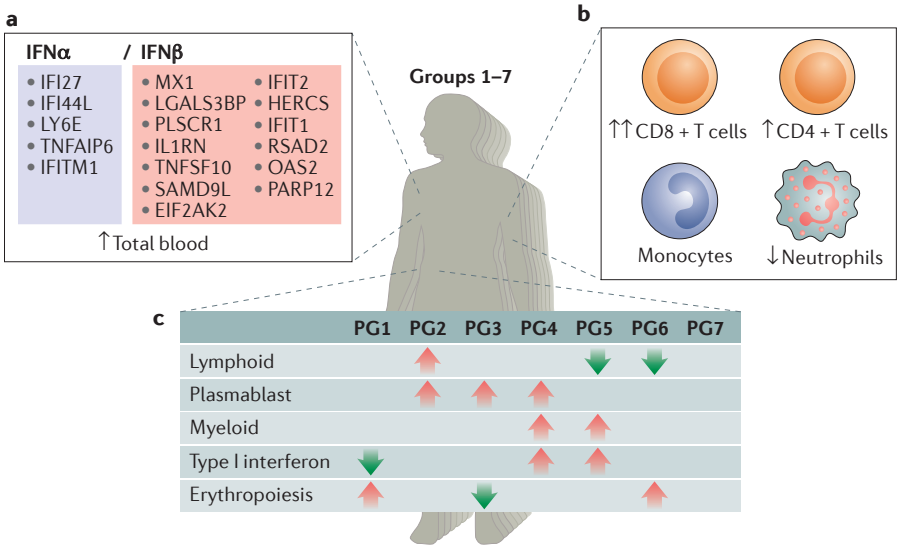

Figure 1 | **Insights into systemic lupus erythematosus (SLE) from gene expression profiling.** **a** | List of differentially expressed genes in patients with SLE (purple; IFNα signature) compared to patients with multiple sclerosis treated with IFNβ (pink; IFNβ signature). **b** | A higher prevalence of type I IFN signalling in T cells than in other immune cell types has been discovered in patients with SLE. **c** | Stratification of patients with SLE into seven patient groups (PG1-7; with different types of cellular mechanisms) based on SLEDAI (SLE Disease Activity Index) correlates. Down arrows represent groups where at least 40% of the individuals have an average correlation of >0.4 with the annotated module space, whereas up arrows represent the top four anticorrelated average values between the identified patient groups and modules.

regarding hypomethylation of type I IFN genes in naive CD4+ T cells[7]) suggests that type I IFN T-cell-signalling might contribute to the development of SLE. In summary, this paper suggests the existence of genes that differentially regulate the type I IFN signalling pathway in what seems to be a cell-type and disease-specific manner.

Banchereau *et al.*[3] identified patient-specific co-expressed gene modules by means of the WGCNA method, using longitudinal gene expression data from paediatric patients with SLE. Interestingly, when these modules were correlated with SLEDAI (SLE Disease Activity Index) and expression profiles were projected on to the Chaussabel modules[6], seven groups of patients were revealed, corresponding to five immune signatures that differed from each other in terms of the types of cellular mechanisms involved: lymphoid, erythropoiesis, plasma cell, neutrophil/myeloid, and type I IFN signatures. An interesting question arises in relation to the study by Flint *et al.*[2], as Banchereau *et al.*[3] found T-cell-associated modules to be underexpressed in patients with SLE.

No differences were observed across the groups in terms of demographic parameters, with the exception of one group in which all patients had lupus nephritis that correlated with the type I IFN, neutrophil and plasmablast-associated modules[3]. The patients in this group had anti-double-stranded DNA antibodies and severe disease. In addition, the neutrophil and type I IFN signatures correlated strongly with development of lupus nephritis and were modified by mycophenolate mofetil treatment, particularly in patients with proliferative rather than membranous glomerulonephritis.

Importantly, grouping patients in this way might serve as a basis for personalized treatment, and could be used for drug repurposing analysis. Stratifying patients could help to identify if there are indeed differential pathophysiological pathways behind the different transcriptional profiles and cell types, and if these pathways can be modulated by specific drugs. However, some questions persist; first, can these groups be validated in adult patients with SLE? Another concern is predictability of the groups without the need for long-term follow-up of patients; correlations with other sources of information (that is, serum cytokine levels) could serve as a basis to identify new biomarkers of transcriptional group prediction.

Nevertheless, this work is the first attempt to stratify patients with SLE using longitudinal transcriptional data[3]. Validation and reproducibility of the data — as well as the use of predictive measurements through the use of simple analysis algorithms — will be of utmost importance before these results can be used in the clinical setting.

Autoimmune diseases have several common features and one of them is the presence, at least in some patients, of a type I IFN signature, for which SLE is the main representative disease. The detailed dissection of this signature and implicated cell populations begins to reveal differences and commonalities across diseases. Multi-omics integrated approaches using systems medicine will enable recognition or even re-classification of autoimmune diseases in molecular terms and redefine the use of existing therapies and the design of new ones.

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SYSTEMIC SCLEROSIS IN 2016

Dermal white adipose tissue implicated in SSc pathogenesis

John Varga and Roberta G. Marangoni

Several strands of new research indicate that skin-specific adipocyte progenitor cells regulate myofibroblasts and skin fibrosis in scleroderma.

The roles, origins and differentiation of intradermal adipocytes, and the contributions of these cells to skin fibrosis, are topics receiving increasing attention in the field of systemic sclerosis (SSc) research. The pathogenesis of fibrosis in SSc remains elusive. Furthermore, although patients with SSc experience fibrosis in multiple organs synchronously, whether fibrosis in different organs is driven by the same or different cells and pathways is unclear. Indeed, the specialized architecture of these organs, and the distinct organ-specific relationships between the vasculature, stroma, lining epithelium and adipose tissue depots, suggest that unique mechanisms, signalling cues and cell–cell communications might underlie organ-specific fibrosis. Several studies published in 2016 have identified dermal white adipose tissue (dWAT) as a new adipose depot and highlight the existence of mechanistic links between cutaneous adipogenesis and fibrogenesis[1–3].

dWAT is a unique adipose tissue that lies within the skin, is distinct from subcutaneous (also called hypodermal) adipose tissue, and seems to be required for skin barrier function. Loss of this tissue, which is a prominent feature of human SSc (FIG. 1) as well as mouse models of the disease, adversely affects skin homeostasis and promotes dermal fibrosis. These new insights, coupled with reports of sustained clinical benefit from adipose-derived stem cell (ADSC) therapy (autologous fat grafting) in SSc, draw attention to the role of white adipose tissue (WAT) in skin fibrosis and could have broad therapeutic implications.

The dWAT story begins in the 1970s, when dermatologist Raul Fleischmajer described atrophy of intradermal adipose tissue and its replacement by collagen-rich fibrous tissue as consistent features of SSc[4], leading him to speculate that fat loss might represent a key pathogenic event. Although this astute observation remains unchallenged, little research has been carried out to elucidate the nature and significance of dermal fat loss. During the past 5 years, atrophy of dermal adipose tissue accompanying skin fibrosis has been highlighted in various mouse models of SSc. Time-course studies established that loss of dermal adipocytes, coupled with downregulation of adipocyte markers such as fatty acid-binding protein 4 (FABP4), peroxisome proliferator-activated receptor γ2 (PPARγ2) and adiponectin in the skin, actually preceded histological or biochemical evidence of fibrosis, suggesting that dWAT involution represents an early and possibly primary event in dermal fibrosis[5].

WAT is made up of unilocular adipocytes and the so-called stromal vascular fraction, comprising ADSCs. Adipocytes store energy and secrete adipokines, whereas ADSCs have regenerative capacity and give rise to adipocytes and other mesenchymal lineages. WAT is organized into distinct depots throughout the body. In mice, dWAT is readily recognized as a discrete layer of fat cells situated between the dermis and the panniculus carnosus muscle, whereas in humans (who lack panniculus carnosus), dWAT is found in pilosebacious units called dermal cones[6]. Like other WAT depots, dWAT is rich in progenitor cells that give rise to adipocytes or dermal reticular fibroblasts via cell-fate decisions regulated by PPARγ. Indeed, pharmacological PPARγ activation in mice promotes systemic adipogenesis, including dermal adipose tissue expansion, at the expense of skin fibrosis[7]. Intradermal adipocytes display unusually rapid growth kinetics, which explains the ability of dWAT to undergo swift expansion (hyperplasia) in response to cold stress or bacterial challenge, as well as during wound healing and the hair cycle[8]. In view of the growing recognition of the importance of dWAT in skin barrier function, and its atrophy in skin fibrosis, there is considerable interest in approaches to measure and visualize this depot.

In 2016, Kasza *et al.*[1] described non-invasive MRI-based imaging strategies for quantitating dWAT in mice and humans. Using MRI, the authors were able to visualize dWAT and demonstrate a strong correlation between dWAT thickness determined by imaging and by direct histologic assay. The MRI technique also demonstrated expansion of dWAT in mice treated with a high-fat diet, highlighting the sensitivity of this approach to monitoring change and its potential utility for longitudinal studies. A similar imaging strategy was applied to ten healthy human subjects in a small pilot study. The results showed that skin-associated fat was highly variable and showed no correlation with BMI or sex. Interestingly, a correlation between the thickness of skin-associated fat with self-reported temperature preference was noted, with those preferring warmer temperatures paradoxically having a thicker layer of fat[1].

What triggers adipocyte attrition and dWAT involution during skin fibrosis, and how might this process contribute to pathogenesis in SSc? To address these questions Mastrogiannaki *et al.*[2] generated transgenic mice in which platelet-derived growth factor receptor α-positive (PDGRα$^+$) progenitor cells expressed constitutively active ß-catenin. The Wnt–ß-catenin pathway is of particular interest as it

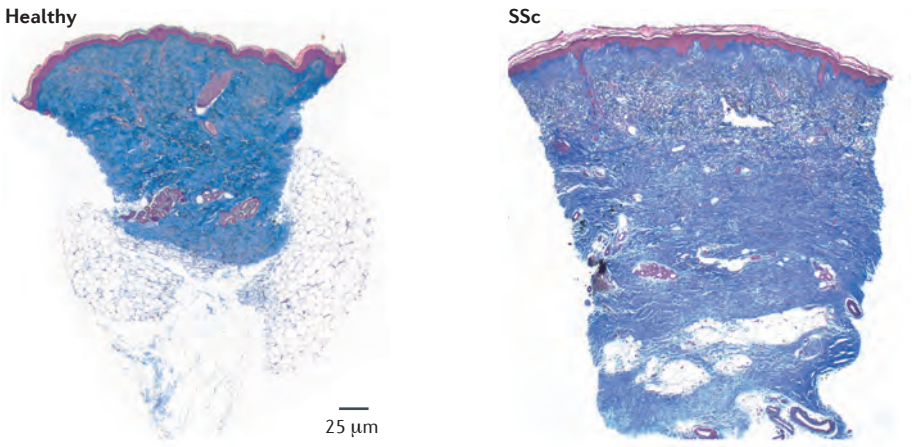

Figure 1 | **Loss of dermal white adipose tissue in systemic sclerosis (SSc).** Replacement of dermal white adipose tissue by fibrous matrix is apparent in a tissue sample (obtained by punch biopsy) from a patient with diffuse cutaneous SSc (right), as compared with that from a healthy individual (left).

Key advances

- Novel MRI imaging approaches can accurately quantitate intradermal adipose tissue in mice and in humans[1]
- Expression of Wnt–ß-catenin in fibroblast progenitor cells disrupts adipocyte differentiation and permits emergence of fibrotic cells in the skin[2]
- Adipocyte progenitor cells within the dermis are required to limit fibrosis, are lost during the development of skin fibrosis, and depend on local dendritic cells for their continued survival[3]

is implicated in SSc pathogenesis and drives fibroblast activation while blocking adipocyte differentiation. The transgenic mice expressing ß-catenin in PDGRα$^+$ progenitor cells showed attrition of the dWAT layer and a corresponding increase in fibrotic dermis[2]. *Ex vivo*, stem cell antigen-1-positive (SCA1$^+$) fibroblast progenitor cells expressing constitutively active ß-catenin showed impaired adipocyte differentiation. These observations are consistent with earlier reports demonstrating attrition of dWAT and its replacement by fibrotic tissue in transgenic mice expressing Wnt10b in FABP4$^+$ progenitor cells[9], indicating that suppression of adipogenesis by Wnt–ß-catenin promotes emergence of fibrotic fibroblasts and replacement of dermal adipose tissue with fibrotic tissue. As PPARγ is the master regulator of adipogenesis and required for adipocyte differentiation, it seems likely that the suppression of PPARγ by ß-catenin accounts for the switch from adipocytic to fibroblastic cell fate in the skin. Comparable adipocytic–fibrogenic cell-fate switches can occur during fibrosis in hepatic stellate cells, dermal adipocytes and lung lipofibroblasts, indicating that this phenomenon is not restricted to the skin[5,9,10]. It is reasonable to speculate that cirrhosis, SSc and pulmonary fibrosis in humans might similarly be associated with an adipocyte-to-fibroblast cell-fate switch.

Further insights into the fate of dermal progenitor cells in fibrosis have come from an elegant study by Lu and colleagues[3]. These investigators defined a population of SCA1$^+$ ADSCs within dWAT in mice and showed that their numbers markedly declined during skin fibrosis. Intradermal adipose atrophy was paralleled by an increase in dermal thickness and accumulation of SCA1$^+$ myofibroblasts. Survival of the remaining ADSCs in fibrotic skin was maintained by CD11b$^+$ dendritic cells via lymphotoxin-ß and downstream integrin activation. Moreover, when mice with skin fibrosis were injected intradermally with ADSCs in combination with lymphotoxin-ß receptor stimulation, the injected ADSCs showed enhanced engraftment and survival, and dWAT loss and dermal fibrosis were ameliorated[3].

These findings focus attention on dWAT as a newly recognized, distinct adipose depot that is relevant to skin biology, and suggest that intact dermal adipogenesis and healthy dWAT are required to restrain skin fibrosis. Important questions about the anti-fibrotic role of 'normal' dWAT remain to be clarified; for instance, whether it is attributable to secreted adipokines that act via paracrine mechanisms to inhibit myofibroblast differentiation, to the preferential differentiation of intradermal ADSCs into anti-fibrotic adipocytes rather than fibrotic myofibroblasts, to the maintenance of the population of regenerative and reparative ADSCs within dWAT, or — most likely — to a combination of these factors. Nevertheless, these reports open a new door for exploring skin fibrosis in SSc and related conditions, and might have implications for fibrosis in the liver and the lung. Moreover, these studies suggest novel targets for preventing or reversing fibrosis, including pharmacological enhancement of PPARγ function and other strategies to disrupt the differentiation of ADSCs into fibrotic myofibroblasts.

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Competing interests statement
The authors declare no competing interests.

MICROBIOME IN 2016

T follicular helper cells and the gut microbiome in arthritis

Veena Taneja

Rheumatoid arthritis is associated with an expansion of certain gut commensals, although the underlying mechanism remains unknown. In 2016, studies using experimental models of arthritis have begun to unravel the links between the gut microbiota, T follicular helper cells and arthritis.

The idea that antibodies provide protection against infectious disease dates back more than a century, when infections in animal models were treated with passive antibody therapy. However, the production of antibodies to self-proteins characterizes many autoimmune diseases, including rheumatoid arthritis (RA). The formation of germinal centres (GCs) and interaction with T helper cells are crucial for B-cell activation and the production of antibodies to aid the clearance of infections. The gut — with help from diverse gut microbiota — has an important role in this process; maintaining homeostasis and protecting the body from pathogens by secreting IgA antibodies. Studies of RA in humans and experimental models of arthritis have revealed an association between RA and gut microbiota[1,2],

providing impetus for researchers to define the mechanisms involved. Research from 2016 unveiled a requirement for gut microbiota in the differentiation of T follicular helper (T_{FH}) cells, leading to GC formation, autoantibody production and development of experimental arthritis.

The K/B×N mouse model represents a well-studied model of antibody-mediated autoimmune arthritis that is dependent on gut microbiota, particularly segmented filamentous bacteria (SFB)[3]. Type 17 T helper (T_H17) cells and autoantibodies are thought to have important roles in this and many other experimental models of arthritis. However, treatment with anti-IL-17 monoclonal antibodies in patients with RA has not shown much efficacy[4], prompting research efforts to define the role of other types of T cells in RA pathogenesis. In 2016, Teng and co-workers[5] demonstrated that T_{FH} cells are required for SFB-dependent arthritis in a K/B×N model. T_{FH} cells express CXC-chemokine receptor (CXCR) 5, B-cell lymphoma 6 protein (BCL6), inducible T-cell co-stimulator, programmed cell death protein 1, the cytoplasmic adaptor protein SH2 domain-containing protein 2A (also known as SLAM-associated protein (SAP)) and cytoplasmic IL-21. Adoptive transfer of T cells from a *Cxcr5*$^{-/-}$ mouse into *Tcra*$^{-/-}$ (T-cell-deficient) B×N mice caused the development of mild arthritis, regardless of whether the *Tcra*$^{-/-}$ B×N mice were colonized with SFB or not[5]. The development of mild arthritis in these mice was attributed to the inability of T_{FH} cells from *Cxcr5*$^{-/-}$ mice to migrate to systemic lymphoid tissues. Interestingly, the presence of SFB was required for the differentiation of T_{FH} cells in Peyer's patches and the development of arthritis, as SFB-negative mice did not develop arthritis.

Teng *et al.*[5] further showed that SFB-induced T_{FH} cells in Peyer's patches exit the gut and produce antigen-specific antibodies systemically, which are essential for the development of arthritis. However, cognate T-cell receptor recognition of SFB antigens was not required for SFB-mediated differentiation of T_{FH} cells in Peyer's patches, suggesting a non-antigen-specific role for SFB. The work of Teng *et al.*[5] provides evidence that the induction of T_H17 and T_{FH} cells occurs before the onset of experimental arthritis, which is important in relation to RA in humans as preclinical systemic autoimmunity proceeds the clinical onset of RA. Although much of the work on animal models of arthritis supports a causal role for T_H17 cells in the onset of disease, this study showed that T_{FH} cells are also important[5]. Notably, IL-6 is required for the induction of both T_H17 and T_{FH} cells, and targeting IL-6 in patients with RA has been a successful approach.

In another study from 2016, Block and co-workers[6] confirmed a role for gut microbiota in the differentiation of T_{FH} cells and the formation of GCs, as the depletion of gut microbiota in mice by the use of antibiotics reduced the number of T_{FH} cells and levels of antibody production. In this study, T_{FH} were shown to have a more crucial role in arthritis development than T_H17 cells. IL-17-deficient K/B×N mice developed arthritis and had normal GCs and T_{FH} cells; however, the presence of SFB was required for arthritis development as treatment with antibiotics prevented the development of arthritis in both IL-17-deficient and IL-17-sufficient mice. Transfer of splenocytes from mice that lacked BCL6 (a transcription factor necessary for the differentiation of T_{FH} cells) in their CD4$^+$ T cells into mice lacking αβ T cells led to low levels of antibody production, but no arthritis[6]. These results highlight the contribution made by T_{FH} cells in helping B cells during antibody production and arthritis development. This study[6], in contrast to the work of Teng *et al.*[5], suggests that T_H17 cells are dispensable in a K/B×N model if the right intestinal microbiota is available to drive the differentiation of T_{FH} cells. The differences in the results of these two studies could be caused by the timing of the experiments, as Teng *et al.* looked at that presence of T_H17 cells before the onset of arthritis[5]. It is possible that, in the presence of IL-6, gut T_H17 cells could differentiate into T_{FH} cells in this model, leading to the development of GCs as previously suggested[7].

T_{FH} cells are a heterogeneous population; although both studies discussed above[5,6] demonstrate critical roles for T_{FH} cells in arthritis development, the exact population that is required was unknown. Chevalier *et al.*[8] determined which population of T_{FH} cells (CXCR5$^+$ or SAP$^+$) was required for autoantibody production and development of arthritis in the K/B×N model. Adoptive transfer of CD4$^+$ T cells from SAP-deficient KRN mice, but not those from CXCR5-deficient KRN mice, into B×N mice led to abrogation of antibody production and arthritis. Although these observations suggest a requirement for T cell–B cell interactions during inflammation, non-cognate activation of CD4$^+$ T cells could also help to cause arthritis. CD4$^+$ T cells from *Cxcr5*$^{-/-}$ KRN mice transferred into *Cd28*$^{-/-}$ mice did not cause arthritis, whereas co-transfer of CD4$^+$ T cells from *Cxcr5*$^{-/-}$ KRN mice with CD4$^+$ T cells from complete Freund's adjuvant-immunized *Cd28*$^{+/+}$.*A*$^{g7+/-}$ mice induced GC formation, antibody production and arthritis in *Cd28*$^{-/-}$ mice[8]. These observations suggest that environmental factors or infections might cause non-cognate CD4$^+$ T-cell stimulation, leading to GC formation and the production of autoantibodies. Although Chevalier *et al.*[8] did not study the role of the microbiome, the presence of non-cognate

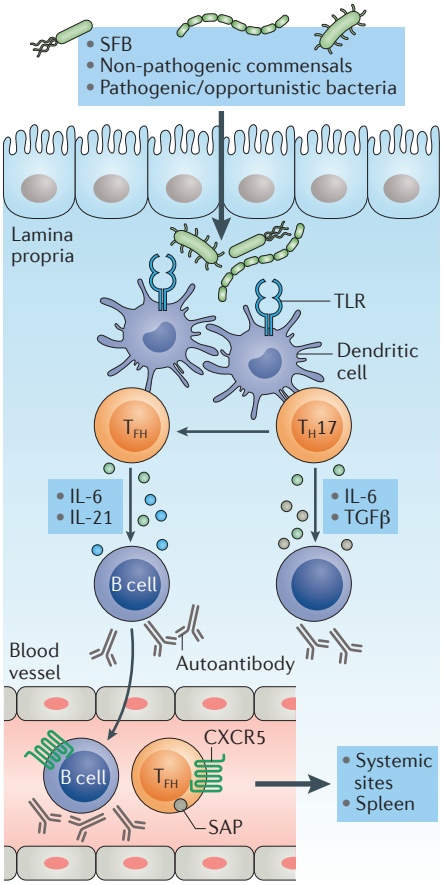

Figure 1 | **Gut microbial diversity in an individual determines the differentiation of T cells into T_H17 or T_{FH} cells.** Dysbiosis might cause an increase in gut epithelial permeability, enabling the translocation of luminal contents and microbial products into the lamina propria. This translocation can lead to activation of the immune system via cognate and non-cognate presentation of microbial products, and the production of various cytokines (such as IL-17, IL-6, transforming growth factor-β (TGFβ) and IL-21). Type 17 T helper (T_H17) cells are required for the clearance of pathogens. In the presence of the appropriate cytokines (IL-6 and IL-21), T_H17 cells can differentiate into CXC-chemokine receptor 5 (CXCR5)-expressing T follicular helper (T_{FH}) cells that help B cells to form germinal centres and produce antibodies. CXCR5$^+$ B cells and T_{FH} cells from Peyer's patches migrate away from the gut and systemically produce pathogenic autoantibodies, causing inflammation and immune complex-mediated pathogenesis. Research published in 2016 suggests T_{FH} cells provide a mechanistic link between the gut microbiota and arthritis[5,6,8].

Key advances

- Generation of type 17 T helper (T_H17) and T follicular helper (T_{FH}) cells precedes arthritis development, indicating a role for both cell types; moreover, segmented filamentous bacteria (SFB)-induced T_{FH} cells migrate out of the gut and generate germinal centres, resulting in autoantibody production and arthritis
- Gut microbiota regulate arthritis in the K/B×N model independently of the presence of T_H17 cells, suggesting T_{FH} cells are critical for the development of SFB-driven and antibody-mediated arthritis in this model[6]
- SAP^+ T_{FH} cells are essential for interaction with B cells, formation of germinal centres and antibody production, linking gut microbiota and arthritis development in the K/B×N model; on the other hand, $CXCR5^+$ T_{FH} cells are required for the generation of optimal immune responses but their contribution can be replaced by non-cognate $CD4^+$ T cells activated via infections or other environmental factors[8]

$CD4^+$ T-cell and B-cell activation could be due to endogenous activation by microorganisms or infectious agents. Infections have long been thought to precede the onset of arthritis, but conclusive evidence and mechanisms have eluded researchers. T_{FH} cells could provide a link between the occurrence of infections and preclinical autoantibody production (FIG. 1).

The gut contains the largest number of antibody-producing plasma cells in the human body. One can speculate that bacteria that contain proteins orthologous for autoantigens in various autoimmune diseases might be involved in GC formation and preclinical autoantibody production when T_{FH} cells activate B cells in the gut. Whether these autoantibodies result in autoimmunity still needs to be investigated. It is conceivable that many commensals carry proteins orthologous for human antigens. The question remains why the presence of these commensals does not result in autoimmunity in healthy individuals, or in all individuals who carry genetic susceptibility alleles. One explanation is that T_{FH} cells are stimulated by the gut microbiota to differentiate into regulatory or IL-17/IL-21-producing cells[9]. The studies highlighted in this article[5,6,8] used mouse models of antibody-mediated arthritis. Experimental models that require the adaptive immune system (such as collagen-induced arthritis) and that use animals carrying arthritis-susceptibility genes could provide critical information on the role of T_{FH} cells and the gut microbiota in modulating arthritis. For example, a 2016 study showed that modulation of T_{FH} cells by blocking glucocorticoid-induced TNF receptor ameliorated collagen-induced arthritis in mice[10]. These observations provide an insight into the role of T_{FH} cells, in addition to T_H17 cells, in the pathogenesis of experimental arthritis. The generation of T_{FH} cells and T_H17 cells possibly depends upon environmental conditions or microbiota in individuals with different genetic susceptibilities to disease; however, research from 2016 suggests that we need to look more closely into the role of gut microbiota and T_{FH} cells in our hunt for therapeutic strategies.

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Competing interests statement
The author declares no competing interests.

MYOSITIS IN 2016

New tools for diagnosis and therapy

Ingrid E. Lundberg

In 2016, there have been several major scientific achievements related to myositis, including the discovery of a novel autoantibody and the relationship between autoantibodies and distinct clinical phenotypes. Advances in the way clinical trials are conducted have also led to breakthroughs in treatment strategies.

In the past few years, there have been great achievements in the field of myositis research that are shedding new light on the pathophysiology of this disease. Last year, yet another new myositis-specific autoantibody (MSA) was identified[6] and clinical phenotypes such as treatment response and tissue sample features began to be characterized in relation to a patient's autoantibody profile. Other important steps forward were the results from controlled trials using conventional immunosuppressive therapy in patients with juvenile dermatomyositis[7] and looking at the clinical and molecular effects of exercise in combination with immunosuppressive therapies in patients with adult myositis[8].

Myositis is a heterogeneous group of disorders that mainly affect the muscles, resulting in muscle weakness. The standard subclassifications of polymyositis, dermatomyositis and inclusion-body myositis (IBM) do not satisfactorily distinguish between clinically or histopathologically distinct subgroups of patients. MSAs, on the other hand, are associated with distinct clinical phenotypes and represent an opportunity to improve

Key advances

- Myositis-specific autoantibodies, such as anti-FHL1 antibodies, can be used to stratify patients into distinct clinical phenotypes with clearly defined histopathology[5]
- Combined therapy with glucocorticoids and either methotrexate or ciclosporin is more favourable than treatment with glucocorticoids alone for patients with juvenile dermatomyositis[6]
- Exercise could be included in treatment regimens alongside conventional therapy without adverse effects[8]

classification criteria (FIG. 1). For example, anti-melanoma differentiation-associated gene 5 (MDA5) antibodies are associated with clinical amyopathic dermatomyositis and interstitial lung disease[1].

To address the question of whether prognosis varies between patients with different MSA subtypes requires large cohorts of well-characterized patients, followed longitudinally. In 2016, Pinal-Fernandez and colleagues[2] utilized one such cohort of patients with myositis, comparing patients with anti-signal recognition particle (SRP) antibodies and those with anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) antibodies, both of which are associated with necrotizing myopathy[3]. In this cohort, anti-SRP antibodies were associated with more-pronounced muscle weakness and worse prognosis for muscle performance compared with anti-HMGCR antibodies[2]. Only 50% of 37 anti-SRP-positive patients reached full or near-full strength after 4 years of treatment in a specialist centre for muscle diseases, with young age at onset being a predictor of poor response to treatment[2]. Differences in the pattern of muscle involvement, as identified by MRI, between patients with anti-SRP and anti-HMGCR antibodies further supports the role of MSAs in subgrouping patients[3]. Muscle involvement in patients with anti-SRP and anti-HMGCR antibodies differed in the location of inflammation in thigh muscle groups and by severity, as measured by degree of oedema, muscle atrophy and fatty replacement[3]. Patients with anti-SRP antibodies had more-severe involvement of muscles compared with patients with anti-HMGCR antibodies[3].

The MRI-identified differences in muscle involvement seen in patients with distinct MSAs suggest that there could be differences in molecular disease pathways in these subgroups of patients. Different histopathological features are associated with different MSAs, adding evidence to this theory. Following this pattern, muscle biopsies from patients with anti-Jo-1 antibodies (which are directed against histidyl-tRNA synthetase) are characterized by perimysial and perivascular inflammatory infiltrates and perifascicular necrosis and atrophy[4]; and muscle tissue samples from patients with anti-transcription intermediary factor 1γ (TIF1γ; also known as E3 ubiquitin-protein ligase TRIM33) antibodies have more signs of mitochondrial dysfunction than anti-Jo-1-positive patients[5].

Notably, the MSAs discussed so far target ubiquitously expressed cytoplasmic antigens, several of which are involved in protein synthesis. The association between these MSAs and the localization of inflammation to skeletal muscles needs further clarification, but the upregulation of some of these autoantigens in regenerating muscle fibres makes such fibres a possible target for the immune system. However, whether MSAs have a direct role in causing muscle weakness or muscle inflammation remains to be seen.

Towards the end of 2015, an MSA, anti-four and a half LIM domains protein 1 (FHL1), was discovered by screening a muscle complementary DNA library with sera from patients with myositis[6]. Mutations in *FHL1* are associated with severe muscle dystrophies in patients, leading Albrecht *et al.*[6] to select anti-FHL1 antibodies for further investigation. Interestingly, anti-FHL1 antibodies were present in ~25% of patients with myositis (dermatomyositis, polymyositis and IBM), but not in those with other autoimmune disorders or muscular dystrophies[6]. These autoantibodies were associated with a clinical phenotype characterized by muscle atrophy, severe muscle weakness, dysphagia and poor response to immunosuppressive treatment. Anti-FHL1-positive individuals also had a more severe histopathological pattern overall, compared with anti-FHL1-negative patients with myositis. Expression of FHL1 was patchy, being seen in aggregate formations in tissue samples from anti-FHL1-positive patients, compared with a homogenous pattern of expression in antibody-negative patients or healthy individuals[6]. Work in animal models provided evidence for a pathogenic role for anti-FHL1 antibodies in muscle inflammation as myositis-prone mice developed more-severe muscle weakness and histopathology than control mice after immunization with the FHL1 protein[6], but the role of anti-FHL1 antibodies in human myositis needs further exploration.

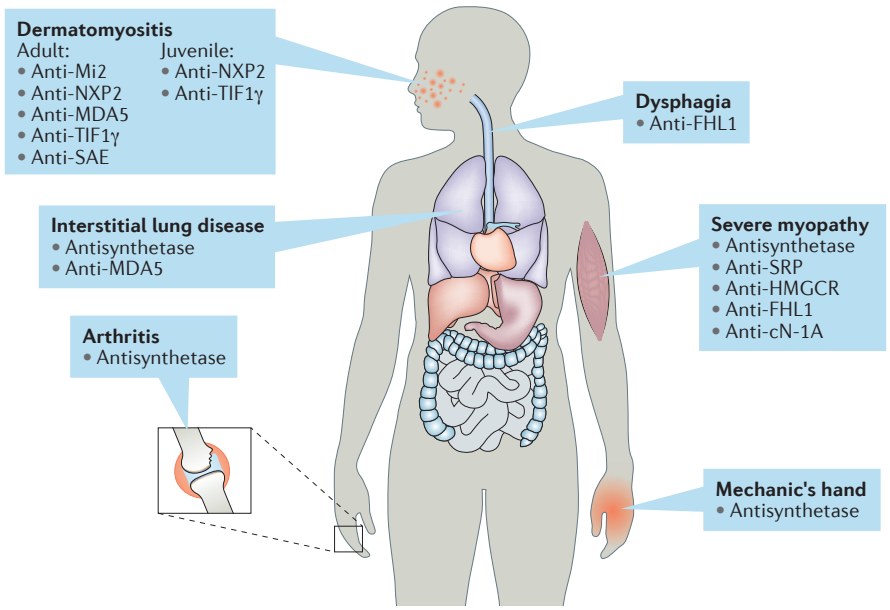

Figure 1 | **Myositis-specific autoantibodies in adult and juvenile myositis.** Myositis-specific autoantibodies can be subgrouped according to their association with clinical phenotypes. For example, antisynthetase antibodies are associated with myositis, interstitial lung disease, mechanic's hand and arthritis, whereas anti-melanoma differentiation-associated gene 5 (MDA5) antibodies are associated with interstitial lung disease and dermatomyositis. Anti-signal recognition particle (SRP) antibodies and anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) antibodies are associated with necrotizing myopathy, anti-four and a half LIM domains protein 1 (FHL1) antibodies with muscle atrophy, dysphagia and inflammation in the muscle, and anti-cytosolic 5′-nucleotidase 1A (cN-1A) antibodies with inclusion body myositis. Mi2, chromodomain-helicase-DNA-binding protein 3; NXP2, MORC family CW-type zinc finger protein 3; SAE, SUMO-activating enzyme; TIF1γ, transcription intermediary factor 1γ.

Treatment of myositis is centred on immunosuppressive therapy, often with disappointing results, leaving many patients with residual muscle impairment and reduced quality of life. Few controlled studies have been performed, and methotrexate, ciclosporin and azathioprine (drugs often used in combination with glucocorticoids) have shown no advantage over glucocorticoids alone in adult patients. In light of previous trials, an investigator-initiated trial in new-onset juvenile dermatomyositis published in late 2015 represents a landmark in the treatment of myositis[7]. This international, multi-centre trial was strongly supported by paediatric investigators around the world. The study included 139 patients with juvenile dermatomyositis and clearly demonstrated the superiority of glucocorticoids in combination with methotrexate or ciclosporin, compared with glucocorticoids alone, on the primary outcome (PRINTO20 response criteria)[7]. In addition, combination therapy gave a shorter time to inactive disease. Overall, the combination of glucocorticoids and methotrexate had a more favourable safety profile than glucocorticoids and ciclosporin. This study emphasizes the need for international collaboration when working with rare diseases like myositis, and will hopefully form the basis of future treatment guidelines.

For a long time, exercise was discouraged for patients with myositis due to fears that it would worsen inflammation. However, evidence is accumulating that exercise is safe for patients with myositis. In 2016, Munters and colleagues published a clinical trial that showed that exercise has beneficial effects on clinical disease activity and aerobic fitness, as well as affecting muscle tissue on a molecular level[8]. The molecular effects of exercise, investigated by use of gene expression analysis and proteomics, included an anti-inflammatory response and the promotion of protein synthesis and mitochondrial activity. This research emphasizes the importance of combining immunosuppressive treatment with exercise and implies that mechanisms other than immune-mediated muscle fibre loss might contribute to muscle weakness, and could be reversed by physical activity[8], although the optimal form of exercise still needs to be determined.

Advances in myositis this past year have been made possible by standardized, longitudinal follow-up studies of large patient cohorts, which are necessary for the identification of differences between antibody-associated subgroups of patients[2,3,5]. The PRINTO network for juvenile dermatomyositis is another great example of international collaboration leading to a large clinical trial[7]. For researchers interested in adult or juvenile myositis, the International Euromyositis Register[9] is a multidisciplinary international collaboration that invites investigators to participate in research projects and to use the electronic register in the clinic. Such registers are likely to contribute to scientific achievements in the future.

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OSTEOARTHRITIS IN 2016

Anti-NGF treatments for pain — two steps forward, one step back?

Nancy E. Lane and Maripat Corr

Inhibitors of β-nerve growth factor (NGF) have impressive effects in reducing musculoskeletal pain, but have also been associated with adverse events of unclear aetiology. Several studies in the past year have sought to clarify the relative benefits and risks of anti-NGF treatment.

Chronic musculoskeletal pain remains a substantial challenge in clinical medicine despite the availability of a number of pharmacologic and non-pharmacologic treatments. In the past 10 years, attention has been focused on nociceptive pain, and in particular on β-nerve growth factor (NGF), a neurotrophin that is needed for normal development of the sympathetic nervous system and the sensory neurons responsible for nociception and temperature sensation. In individuals with musculoskeletal pain, treatment with NGF inhibitors can produce impressive improvements in joint pain and physical function; however, mild neurologic adverse events and cases of accelerated arthropathy have been reported in clinical trials, leading to suspension or delay of investigations into these agents. Both preclinical and clinical studies are ongoing to assess the mechanisms and effects of this novel effective analgesic treatment, and 2016 saw the publication of some notable results in this area.

NGF binds to two receptors on peripheral nociceptors: the high-affinity tyrosine kinase receptor tropomyosin-related kinase A (TrkA) and the low-affinity neurotrophin receptor p75NTR. Binding of NGF to these receptors then activates a signalling pathway of intracellular kinases, which can eventually lead to neurite outgrowth and sensitization of the neurons[1]. In adults, injection with NGF results in pain, a finding that motivated the development of a NGF antagonist approach for clinical pain modulation (FIG. 1).

Key advances

- Adjudication of adverse events observed in clinical trials of the β-nerve growth factor (NGF) inhibitor tanezumab determined that the small number of cases of rapidly progressive osteoarthritis (OA) were particularly associated with high doses of tanezumab or co-administration with NSAIDs[4]
- A large phase II–III study of the high-affinity NGF inhibitor fasinumab showed this agent was effective and well-tolerated in patients with moderate to severe OA of the knee or hip[5]
- Clinical development of fasinumab continues, with careful consideration of OA disease severity and doses being evaluated[6]

Studies of NGF inhibition with the monoclonal antibody tanezumab began around 10 years ago: in a phase I study, patients with knee osteoarthritis (OA) reported a significant and prolonged reduction in knee pain[2]. This work was followed by a phase II trial published in 2010 that demonstrated both the efficacy and safety of tanezumab in individuals with moderate to severe knee OA[3]. Subsequent phase III studies in patients with painful knee and hip OA assessed the safety and efficacy of tanezumab (alone or in combination with NSAIDs), and found that anti-NGF treatment significantly reduced joint or back pain compared with both placebo and NSAIDs[2,3]. However, reported adverse events from these clinical trials included osteonecrosis and rapidly progressive OA of the hip, knee and shoulder. The sponsors found that these adverse joint-related events occurred more frequently in patients receiving high-dose tanezumab and concurrent NSAIDs[4]. Concerns about joint safety led the FDA to put a partial clinical hold on all anti-NGF trials for 26 months (except terminal cancer pain trials), and for an additional 27 months owing to sympathetic nervous system safety concerns.

Fast forward to 2016 and new clinical trial data have become available for another anti-NGF antibody, fasinumab, which is in development for the treatment of chronic pain states including OA and low back pain[5]. A large phase II–III study with fasinumab, in patients with either knee or hip OA who had inadequate pain relief or who could not tolerate standard therapies ($n = 419$), found that fasinumab (at a dose of 1 mg, 3 mg, 6 mg or 9 mg) reduced joint pain by >50% and was statistically significantly better than placebo. Adverse effects reported were those expected from other anti-NGF therapies[5]. During the study period, no cases of osteonecrosis were reported; one case of a subchondral insufficiency fracture in the placebo group plus six cases in the combined fasinumab groups were reported, and one case of rapidly progressive OA was observed in each of the 3 mg, 6 mg, and 9 mg fasinumab dose groups. A phase IIb study of fasinumab for chronic low back pain was also initiated; however, with nearly 70% of the study participants enrolled, a case of arthropathy was observed in a patient in the high-dose (9 mg) fasinumab group who had advanced OA at the time of enrolment. On the basis of this adverse event, the FDA placed the chronic back pain study on partial clinical hold[6].

The results of both tanezumab and fasinumab studies in knee or hip OA and chronic low back pain demonstrate the efficacy of NGF inhibition in reducing pain; however, there seems to be a small group of patients who go on to develop advanced arthropathies, and this effect is most prevalent with higher doses of the biologic agents, or with the combination of NSAIDs and tanezumab.

A number of theories have been proposed to explain the incidence of advanced arthropathies in this setting, the most common being that improved analgesia leads to increased loading of a diseased joint, resulting in disease progression. Alternatively, this phenomenon could be similar to neuropathic arthropathy (Charcot joint), which occurs secondary to loss of sensation in an extremity and can result in joint dislocations, fractures and fragmentation. However, there might be another biological explanation. NGF levels are high in inflammatory tissues, and these tissues have a high number of monocytes. NGF is reportedly part of an important regulatory pathway in monocytes and other cell types[7]. Inflammatory stimuli activate Toll-like receptors (TLRs) on monocytes, which increases expression of the NGF receptor TrkA. Then, NGF binding to TrkA seems to block the TLR responses, and reduces production of inflammatory cytokines (such as IL-1β, TNF, IL-6 and IL-8) while increasing the release of anti-inflammatory mediators (IL-1 receptor antagonist and IL-10). In TLR4-activated monocytes TrkA inhibitors reduced the anti-inflammatory effect of NGF, and thereby increased NF-κB pathway activation and inflammatory cytokine production while decreasing IL-10 production. Other TLRs also increase NGF production[8], and NGF signalling might abate TLR2-mediated cell death[9], broadening the beneficial effects of NGF.

Interestingly, this regulatory pathway might be dysfunctional in inflammatory arthritis. Mononuclear cells from the synovial fluid of patients with juvenile idiopathic arthritis (JIA) had reduced TrkA expression, and NGF administered to lipopolysaccharide-stimulated JIA synovial fluid monocytes and

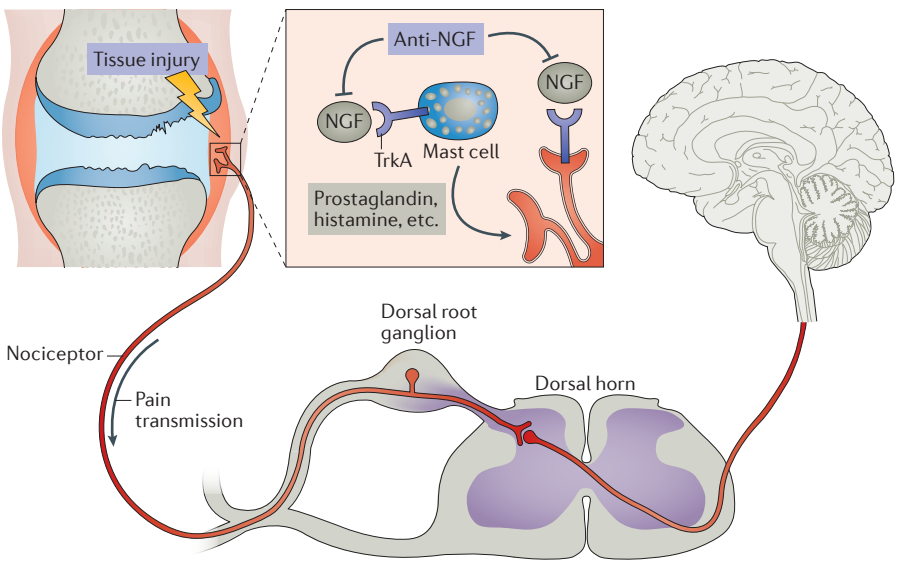

Figure 1 | **Transmission of pain by NGF.** The neurotrophin β-nerve growth factor (NGF) is released upon injury and causes pain by activating its receptor (TrkA) on nociceptors and mast cells (which causes the release of inflammatory mediators such as histamine), leading to transmission of pain signals from the periphery to the spinal cord and brain via the dorsal ganglion. Inhibitors of NGF have shown promise in clinical trials of musculoskeletal pain.

peripheral blood mononuclear cells failed to reduce the production of IL-6, unlike in healthy controls[7]. These data are intriguing as they suggest that alterations in TrkA expression or signalling might disrupt endogenous anti-inflammatory mechanisms, thus increasing joint destruction. They also suggest that inhibition of NGF in the presence of inflammation might increase the inflammatory reaction. Additional evidence exists that the p75NTR NGF receptor is also associated with suppressing TLR-induced maturation of dendritic cells[10]. Further studies will be needed to determine if inhibition of NGF and NGF signalling in monocytes might be relevant to the destructive arthropathy observed in a small number of patients enrolled in the anti-NGF studies.

In summary, NGF inhibitors can markedly reduce chronic musculoskeletal pain, but enthusiasm for these compounds has been dampened by a small number of cases of advanced arthropathy in OA joints. The adverse events have not yet been clearly explained, but understanding the multiple biological pathways that are influenced by NGF is critical if we are to continue the clinical development of NGF inhibitors for chronic pain states. At this time, we seem to have taken two steps forward in the treatment of chronic pain associated with OA, but one step back.

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Competing interests statement
N.E.L. declares that she is a consultant to and advisory board member for Regeneron. M.C. declares no competing interests.

BLADDER DYSFUNCTION IN 2016

New insights into interstitial cystitis and chronic pelvic pain syndromes

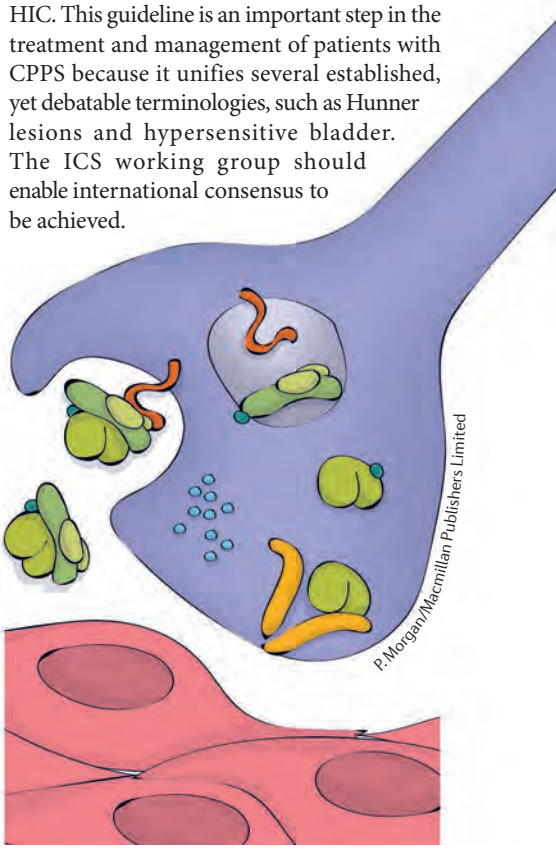
Jia-Fong Jhang and Hann-Chorng Kuo

In 2016, immunohistochemical evidence revealed major differences in the inflammatory characteristics of Hunner and non-Hunner interstitial cystitis/bladder pain syndrome (IC/BPS). Evidence has also emerged that an isomer of testosterone, etio-S, might be a urinary biomarker of IC/BPS. Intravesical botulinum toxin injections became a standard treatment of IC/BPS. Furthermore, the International Continence Society has published a new Standard for Terminology for Chronic Pelvic Pain Syndromes.

Chronic pelvic pain syndrome (CPPS) is a complex and heterogeneous syndrome characterized by a wide range of clinical manifestations. This syndrome, characterized by pain in the pelvic area, potentially includes urological, gastrointestinal, musculoskeletal and/or gynaecological aetiologies. 2016 was an exciting year for research into CPPS, especially into interstitial cystitis/bladder pain syndrome (IC/BPS). Data from several studies have provided novel insights into pathogenesis, terminologies, biomarkers and treatments of CPPS and IC/BPS.

Bladder inflammation, dysfunction of the urothelial barrier and increased expression of sensory proteins are all suggested to account for IC/BPS, although conclusive evidence is available for none of these hypotheses. Inflammation of the bladder has been considered a cornerstone of the pathogenesis of IC/BPS for approximately 100 years; however, the specific inflammatory characteristics have never been fully defined. Maeda and coauthors[1] used immunohistochemical staining and novel image-analysis software (Tissue Studio v.3.5) to quantify the extent of urothelial inflammation in patients with Hunner-type or non-Hunner-type IC/BPS (HIC and NHIC, respectively). This study revealed the presence of significantly more severe lymphoplasmacytic infiltration in the urothelium in patients with HIC than in those with NHIC. Furthermore, the plasma-cell ratio was significantly higher in patients with HIC than in those with NHIC or bacterial cystitis. In the bladders of patients with HIC, the severity of inflammation at the bladder ulcer site was not significantly different to that at the non-ulcer site. In addition, expansion of the light-chain-restricted B-cell population was observed in 31% of patients with HIC versus none with NHIC. The presence of focal clonal B-cell expansion usually suggests an early, minute, mucosa-associated lymphoid tissue lymphoma. These findings indicate that HIC should be considered as a form of pancystitis, and as a distinctly different disease from NHIC. This observation also implies that partial cystectomy of the Hunner lesion alone might not effectively eliminate bladder inflammation in patients with HIC. However, in this study, the correlation between infiltration of inflammatory cells and clinical symptoms, such as a reduction in bladder capacity, was not significant in patients with HIC. Thus, further investigation, to evaluate the role of different inflammatory cells (such as mast cells) in the bladders of patients with IC/BPS is necessary. In terms of genetic characteristics, gene expression analyses were performed on bladder tissue from patients with IC/BPS and from women with stress urinary incontinence without pain[2]. Two inflammation-associated genes, C-C motif chemokine 21 (also known as chemokine ligand 21) and fibroblast growth factor 7, were found to be overexpressed in women with IC/BPS and the level of expression of these proteins was found to be correlated with symptom scores. However, only 15 women with IC/BPS were included in this study, hence further investigation of gene expression patterns in patients with IC/BPS is required.

CPPS is a multifactorial condition, and terminology varies according to the type of specialist responsible for managing the patient. Terms used in the field of CPPS have been poorly defined for some time; researchers might, therefore, be confused on the most appropriate terminologies, resulting in misunderstanding during discussions. In August 2016, the International Continence Society (ICS) published standards for the terminology relating to CPPS, with the aim of improving the understanding of these syndromes and patient diagnosis[3]. Different types of CPPS are classified into nine clinical domains (lower urinary tract (LUT), female genital, male genital, gastrointestinal, musculoskeletal, neurological, psychological, sexual and comorbidities). Symptoms and signs of each domain are defined in a clear and detailed report. Cystoscopic findings, which were previously of debatable clinical relevance, such as glomerulations and Hunner lesions, are also clearly described. In terms of bladder pain, the ICS working group agreed to distinguish hypersensitive bladder, IC/BPS and IC from HIC. This guideline is an important step in the treatment and management of patients with CPPS because it unifies several established, yet debatable terminologies, such as Hunner lesions and hypersensitive bladder. The ICS working group should enable international consensus to be achieved.



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Key advances

- Lymphoplasma-cell infiltration of the urothelium is significantly more severe, often with focal clonal expansion of B-cell populations, in patients with Hunner-lesion IC/BPS compared with those with non-Hunner IC/BPS[1]
- A new Standard for Terminology was published, classifying chronic pelvic pain syndromes into nine different domains; furthermore, definitions were finally provided for several established, yet debatable terms[3]
- Urinary etio-S, an isomer of testosterone, can be used to differentiate individuals with IC/BPS from those without, with a sensitivity and specificity of 84.7% and 91.2%, respectively[5]
- Data from the first prospective, multicentre randomized controlled trial investigating intravesical botulinum toxin A in patients with IC/BPS have resulted in an upgrade to evidence level three in the AUA guidelines

IC/BPS is a clinical syndrome, and its diagnosis is mainly made on the basis of a characteristic complex of LUT symptoms and exclusions of other potentially confusable diseases such as bladder outlet obstruction or overactive bladder (OAB). Some tests, such as cystoscopic hydrodistention or the potassium sensitivity test are helpful in the diagnosis of IC/BPS but might be too invasive for some patients. Thus, non-invasive biomarkers are necessary to help clinicians to accurately differentiate between IC/BPS and diseases with overlapping symptoms, and to evaluate the extent of disease progression. Among the potential biomarkers of IC/BPS, urinary nerve growth factor (NGF) has attracted much attention since 2009. Urinary NGF levels in patients with IC/BPS are significantly higher before treatment compared with those of age-matched individuals without IC/BPS, and also decrease significantly in patients that respond to treatment with hyaluronic acid[4]. In 2016, Parker and co-workers[5] applied mass-spectrometry-based global metabolite profiling to urine samples from women with IC/BPS and found that levels of etiocholan-3α-ol-17-one sulfate (etio-S), an isomer of testosterone, were significantly higher than in urine samples from women without IC/BPS. A statistically significant correlation was observed between urinary etio-S levels and patients' symptoms, such as pain. This approach also enabled accurate differentiation of patients with IC/BPS from those without, with a sensitivity and specificity of up to 84.7% and 91.2%, respectively. However, the relationship between urinary etio-S levels and IC/BPS has not been investigated. The presence of elevated urinary etio-S might only imply acute stress and might not be completely specific for IC/BPS[6]. Further investigations of urinary etio-s levels in patients with bacterial cystitis and/or OAB are necessary.

The treatment of IC/BPS is difficult and frustrating for many clinicians. The exact pathogenesis of IC/BPS remains controversial, thus urologists are often only able to relieve patients' symptoms rather than cure their underlying disease. Despite many efforts to develop new treatments of IC/BPS in the past decades, few have been proven to be effective in clinical practice. Intravesical botulinum toxin-A (BoNT-A) injections are currently widely used in patients with IC/BPS and are recommended as a standard-of-care treatment in most guidelines. However, the FDA have yet to approve the use of BoNT-A in patients with IC/BPS. Results of the first prospective, multicentre, randomized, double blind, placebo-controlled clinical trial designed to investigate the efficacy of intravesical BoNT-A injections versus normal saline injections in patients with IC/BPS have been published this year[7]. At 8 weeks after treatment, a significantly greater reduction in the severity of pain was observed in the BoNT-A group compared with the placebo group (visual analogue score, -2.6 ± 2.8 versus -0.9 ± 2.2; $P = 0.021$). The overall treatment success rates were 63% (26/40) in the BoNT-A group and 15% (3/20) in the placebo group ($P = 0.028$). This result also elevates the evidence rating of BoNT-A injections as a treatment of IC/BPS from level four to level three in the AUA guidelines.

Previous immunohistochemical evidence indicated that the chronic urothelial inflammation and apoptosis observed in patients with IC/BPS are ameliorated by BoNT-A injections[8]. In September 2016, data were published from a randomized, placebo-controlled trial investigating the efficacy of AQX-1125 — an SH2-containing inositol-5′-phosphatase 1 (SHIP1) activator with anti-inflammatory effects — in patients with IC/BPS[9]. In comparison with the placebo group, patients treated with an oral SHIP1 activator had greater improvements in bladder pain scores and O'Leary-Sant Symptom Index scores. The main adverse events in patients receiving AQX-1125 were diarrhoea (in 10.8% of patients) and skin rash (in 10.8% of patients). Nevertheless, the overall adverse event rates in patients receiving AQX-1125 or placebo were not significantly different (51.4% and 78.1% of patients, respectively). Data from the first randomized controlled trial designed to investigate the use of gabapentin in women with CPPS were also published in 2016 (REF. 10). At 6 months, participants receiving gabapentin had significantly less severe bladder pain and significantly improved mood compared with those receiving placebo. Trials investigating these novel treatments of IC/BPS and CPPS have provided exciting results; however, further clinical trials are required to confirm the reproducibility of these results, including the efficacy and safety. Clearly, a considerable amount of research is required before these treatments can be widely used. Data from laboratory-based studies will also help confirm the validity of these novel approaches.

In 2016, data from studies involving patients with CPPS and/or IC/BPS provided strong new evidence in several different domains. More breakthrough studies of pathogenesis, biomarkers and treatments, based on results from 2016, can be expected in the following years. Researchers and clinicians should closely follow the development of these novel concepts, in order to expand our understanding and provide the best level of care for patients.

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The authors declare no competing interests.

PROSTATE CANCER IN 2016

Improved outcomes and precision medicine come within reach

Cora N. Sternberg and Himisha Beltran

2016 was an important year for prostate cancer research. New clinical data highlight the need for personalized treatment across clinical disease states and have changed clinical practice for men with metastatic disease. Molecular studies have characterized tumour heterogeneity and informed biomarker development for advanced disease and research into mechanisms of treatment resistance.

The management of clinically localized prostate cancer detected by measurement of serum PSA levels is a controversial topic. The ProtecT trial included 82,429 men aged 50–69 years who had a PSA test between 1999 and 2009; 2,264 of these men were diagnosed with prostate cancer and 1,643 men agreed to be randomized to either active monitoring, radical prostatectomy or external beam radiation therapy[1]. ProtecT was intended to address the comparative effectiveness of these three approaches with a primary outcome measure of prostate-cancer-specific mortality (PCSM). Importantly, the majority of patients enrolled in this study had low-risk disease (>75% with Gleason score 6). Although the incidence of disease progression including metastases was higher in patients undergoing active monitoring than in the other groups at a median follow-up period of 10 years — and this follow-up duration might be too short to evaluate PCSM — overall PCSM was remarkably low in all three study arms: only ~1% of patients died from their disease, irrespective of the treatment assignment. ProtecT highlights one end of the prostate cancer disease spectrum, patients with tumours with a low probability of affecting patient mortality, and the importance of individualizing care and discussing treatment options such as clinical monitoring or active surveillance for patients with low-risk disease.

At the other end of the spectrum, in patients diagnosed with advanced prostate cancer, research published in 2016 has shown us that advanced tumours might be better treated with combination therapy strategies, potentially due to intratumoural heterogeneity. The STAMPEDE trial employed an innovative multiarm multistage design that used one control arm and several comparator arms to evaluate therapies beyond the standard of care (SOC) of androgen deprivation therapy (ADT) alone for patients with hormone-sensitive prostate cancer (HSPC)[2]. Investigators from primarily the UK and Switzerland recruited men with high-risk, locally advanced, metastatic or recurrent prostate cancer starting first-line long-term ADT. Men were randomly assigned 2:1:1:1 to four groups, evaluating SOC (ADT alone) versus ADT plus six cycles of docetaxel, zoledronic acid or both. Of a total of 2,962 treated men, 61% had M+ disease, 15% had N+/NxM0 and 24% had N0M0 disease. Of note, only 6% of men had previously been treated with local radiotherapy or radical prostatectomy.

At a median follow-up duration of 43 months, the median overall survival was 71 months for the ADT alone group (32 months to not yet reached) and 81 months (41 months to not yet reached) for ADT plus docetaxel (HR 0.78, 95% CI 0.66–0.93; $P=0.006$)[2], supporting the use of chemohormonal combination therapy for HSPC. No evidence of heterogeneity in treatment effect was found for any of the prespecified subsets. Notably, no survival benefit or decrease in skeletal-related events (SREs) were observed with the addition of zoledronic acid (HR 0.94, 95% CI 0.79–1.11; $P=0.450$), consistent with prior studies and providing further evidence against the routine use of zoledronic acid for SRE prevention in the HSPC disease state.

These results coincide with those reported in the CHAARTED trial from the USA in patients with metastatic HSPC treated with ADT alone or the combination of ADT and six cycles of docetaxel[3]. Patients with high-volume disease who received ADT plus docetaxel had a 17-month benefit in median overall survival compared with those receiving ADT alone (49.2 months versus 32.2 months, respectively). An update, presented at the ESMO Congress 2016, presenting data with 2.4 years of additional follow-up duration, corroborated the positive results in patients with high-volume disease (HR 0.63, 95% CI 0.50–0.79; $P<0.0001$), but patients with low-volume disease had no survival benefit from the addition of docetaxel to ADT (HR 1.04, 95% CI 0.70–1.55; $P=0.86$)[4].

Based on these results and a systematic review and meta-analysis[5], guidelines, such as those from ESMO and EAU, now support docetaxel treatment as a SOC in combination with ADT as first-line treatment of metastatic HSPC in men fit enough for chemotherapy (level of evidence 1, recommendation grade A). NCCN guidelines more cautiously consider this option for fit patients with high-volume disease.

Castration-resistant prostate cancer (CRPC) has a wide range of clinical behaviours. Important gains in survival have been achieved in the past decade and six drugs are now approved for the treatment of patients with metastatic CRPC. However, current treatment decisions are not based on molecular tumour stratification, which might have contributed to the failure of multiple clinical trials.

One landmark study demonstrated that up to 20% of metastatic CRPC tumours harbour genomic alterations that affect DNA repair pathways, most commonly involving *BRCA2* and *ATM*[6]. These findings have promising implications for predicting therapy responses to

Key advances

- The ProtectT trial[1] found remarkably low 10-year overall prostate-cancer-specific mortality (~1%), highlighting the relative indolence of Gleason score 6 disease and the importance of individualizing therapy in this patient group
- STAMPEDE trial[2] data concur with CHAARTED trial[3] data showing that addition of docetaxel chemotherapy to androgen deprivation therapy (ADT) results in extended survival in patients with metastatic hormone-sensitive prostate cancer compared with ADT alone
- Pritchard *et al.*[7] found a high incidence (11.8%) of germline mutations in DNA repair genes in tumours of men with metastatic prostate cancer, which has implications both for treatment considerations and for the disease risk of relatives
- Wyatt *et al.*[8] demonstrated the feasibility of noninvasive sequential testing of circulating tumour DNA to assess genomic alterations in patients with castration-resistant prostate cancer undergoing systemic therapy, paving the way for future biomarker-driven trials
- New insights into treatment resistance mechanisms and tumour heterogeneity, including the neuroendocrine phenotype, were provided by Beltran *et al.*[9] and Kumar *et al.*[10], highlighting the importance of clinical and molecular integration to identify patient subsets with resistant disease

poly(ADP-ribose) polymerase (PARP) inhibitors and, potentially, platinum-based chemotherapy. Unexpectedly, in addition to somatic alterations, a high incidence of germline mutations in DNA repair genes (8%) was also found in that study.

In 2016, these initial discoveries were extended to further define the frequency of germline mutations in patients with metastatic prostate cancer. Pritchard *et al.*[7] evaluated germline DNA of 692 men with metastatic prostate cancer who had not been selected for family history or other clinical features, focusing their analysis on 20 DNA repair genes. In 11.8% of men, 84 predicted deleterious germline DNA repair gene mutations were found, including *BRCA2* (5.3%), *ATM* (1.6%), *CHEK2* (1.9%), *BRCA1* (0.9%), *RAD51D* (0.4%) and *PALB2* (0.4%). Similar to breast cancer, the presence of germline mutations involving DNA repair genes in men with prostate cancer might help identify family members at risk of developing not only prostate cancer but also other cancers. The high prevalence of such mutations in this study suggests that all men with metastatic prostate cancer should be considered for routine genetic testing irrespective of family history or age. These findings also indicate a need for specialists in genetic counselling as an integral part of the multidisciplinary team. The frequency and significance of germline DNA repair defects within specific age, ethnic and racial subgroups require further study.

In addition to these DNA repair alterations, other genomic alterations enriched in metastatic CRPC tumours have potential clinical implications; for example, somatic alterations in *AR* (60%), *TP53* (50%), *PTEN* (40%) and *RB1* (10%)[6]. As metastatic biopsies cannot always be performed serially, researchers have started to evaluate the potential of these alterations in predicting response to therapy and/or prognosis using liquid biopsies including analysis of circulating tumour DNA (ctDNA). One study demonstrated that the relative abundance of individual lesions captured in ctDNA can be detected and used to track clonal subpopulations in the circulation and other studies have since found that the presence of *AR* mutations or amplification in ctDNA is associated with a decreased response to subsequent treatment with abiraterone or enzalutamide. In 2016, one team of researchers used ctDNA analysis to assess and serially follow a large panel of alterations in 65 patients with CRPC during the course of systemic therapy[8]. They found that genomic profiling of ctDNA was feasible in nearly all patients with CRPC and was clinically informative in identifying actionable alterations, demonstrating how emerging technologies could guide the design of future biomarker-driven trials.

Understanding how molecular alterations contribute to treatment resistance is critical. One study published in 2016 evaluated the evolution of CRPC to a neuroendocrine phenotype, an aggressive AR-indifferent subtype of CRPC, showing that alterations in, for example, *RB1* and *TP53* were commonly acquired[9]. By integrating whole-exome, transcriptome and epigenetic data, the team found that these neuroendocrine tumours commonly evolve from a prostate adenocarcinoma precursor through a mechanism of divergent clonal evolution. In another study published in 2016, an examination of the heterogeneity of the lethal phenotype by evaluating spatially distinct metastases in patients at the time of autopsy discovered that both early and key late driver lesions were often shared between metastases[10], suggesting shared vulnerabilities between tumour lesions. The clonal evolution that occurs during the course of therapy, including the development of the neuroendocrine phenotype, and the relative contribution of intratumoural heterogeneity in driving treatment resistance has potential clinical implications. Understanding these mechanisms could help in identifying subsets of patients that might be less likely to respond to AR-targeted therapies and in elucidating mechanisms of response to current and emerging therapeutics.

Overall, these studies presented in 2016 set a foundation for future research and a path towards integration and validation of molecular data in clinical care across multiple disease states. We anticipate that this work will continue to be a major focus of prostate cancer research in 2017.

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Competing interests statement
The authors declare no competing interests.

MICROBIOTA IN 2016

Associating infection and incontinence with the female urinary microbiota

Linda Brubaker and Alan J. Wolfe

The discovery and confirmation of the female urinary microbiota in 2012 provided opportunities to improve insight into lower urinary tract disorders in women, including UTI and urgency urinary incontinence. Now, research in 2016 has shown that expanded culture techniques enable improved uropathogen detection and confirm that bacteria detected by culture-independent methods are alive.

Nearly every clinician assesses urinary health from time to time; for example, ordering and interpreting a urine analysis and/or urine culture to detect a UTI. Clinicians providing care for women with lower urinary tract (LUT) disorders have also relied on these traditional urinary assessments in order to evaluate, diagnose, and treat affected patients. However, all

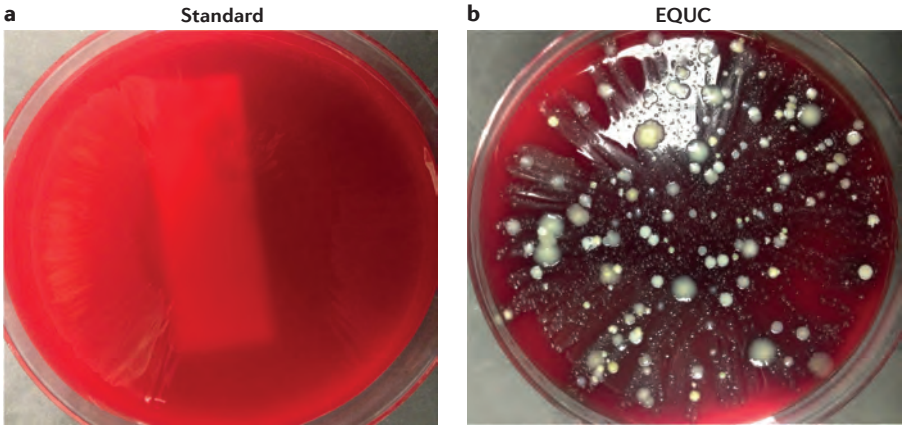

Figure 1 | **Urine is not sterile.** This urine sample was obtained by transurethral catheter from a woman seeking urogynaecology care. **a** | 1 µl urine was spread on a blood agar plate and incubated for 24 h at 35 °C at ambient atmosphere. This technique is part of the standard urine culture protocol. **b** | 100 µl urine from the same patient was spread on a blood agar plate and incubated at 35 °C for 48 h. Increased growth of bacterial colonies can be seen, demonstrating that the urine sample was not sterile, despite being obtained directly via a transurethral catheter. This technique is one of many growth conditions that comprise the enhanced quantitative urine culture (EQUC) protocol. Reprinted with permission from College of American Pathologists. *CAP Today*, Aug. 2016.

of these assessments are based on the assumption that the healthy female urinary bladder is sterile, an assumption that we now know to be incorrect. Since 2012, when microbes were detected in the female urinary bladder (the female urinary microbiota)[1], additional and confirmatory evidence has clearly documented the presence of a living bacterial community in the bladders of women with and without lower urinary symptoms[2–6]. This major paradigm shift raises many questions regarding the role of the female urinary microbiota in lower urinary tract health and disease.

Much more is known about the microbiota of other organs than that of the bladder. For example, the well-studied gut microbiota are known to vary considerably based on dietary intake and BMI. Studies that link the gut microbiota with CNS function highlight the biological importance of microbial niches throughout the human body. The urinary microbiota holds similar potential, especially given the well-known connections between brain and bladder function[7].

Three papers published in 2016 have addressed several clinically relevant questions in the field. Karstens and co-workers[8] provided confirmatory evidence of the presence of bacterial communities in the bladders of women with and without lower urinary tract symptoms. In a carefully controlled study, these investigators collected catheterized urine samples from clinically well-characterized women (mean age approximately 58 years) with urgency urinary incontinence (UUI, $n = 10$) and nine women without such symptoms. The investigators reported detection of relevant bacterial sequences in 95% of samples, and a median DNA content of 95 fg/mL without group differences between women with and without symptoms. They also observed a higher number of operational taxonomic units (OTUs) in samples from women with UUI (mean = 49) compared with those without, although these data were not statistically significant (mean = 39 ($P = 0.2$)), group differences in OTU abundance (In UUI, nine more abundant and five less abundant, compared with controls), and significant individual variation in the number of bacterial families (range 2–49) detected per sample, as well as the diversity and richness of each sample. Overall, they concluded that increased symptom distress and urgency incontinence episodes correlated with the characteristics of the urinary microbial community, which could be clinically important.

The work of Karstens and colleagues[8] provides important and independent confirmation of work previously published by Pearce *et al.*[4] who first described differences in the urinary microbiota of women with UUI compared with those without such symptoms. Despite the small size of Karstens and colleagues' (REF. 8) cohorts, the findings are strengthened by the careful characterization and matching of the participants. In addition, the proportion of samples that were sequenced is high; this strength would have been further enhanced by the concomitant use of enhanced culture-based techniques[2,3,5] to confirm that the detected DNA arose from living microbes.

A complementary paper by Thomas-White and co-workers[9] used pre-existing samples collected in an NIH study designed to evaluate the treatment outcomes of well-characterized women enrolled in a multicentre prospective randomized trial (the Value of Urodynamic Evaluation (ValUE) study) to assess the role of preoperative urodynamic testing before surgery for stress urinary incontinence (SUI). Women with concomitant UUI were included, as clinically appropriate. The study relied on samples (mostly obtained through voiding ($n = 174$), but some obtained through catheterization ($n = 23$)) obtained at baseline, before SUI treatment. Most (86%) of the 197 samples contained detectable levels of bacterial DNA. An important clinical message was that no association with SUI symptoms was observed, in agreement with the fact that SUI and UUI have different aetiologies. Similar to data from previous studies, the community characteristics — including diversity and organism predominance — of the urinary microbiota were associated with UUI symptoms. In addition, the large sample size meant that these investigators were also able to detect an association between hormonal status and BMI. Finally, they demonstrated that increased diversity of the urinary microbial community was associated with a concomitant lower frequency of *Lactobacillus* in clinically postmenopausal women who are not taking exogenous oestrogen.

A third paper by Price and co-workers[5], addressed the clinical relevance of bacterial members of the urinary microbiota, focusing on the ability of the standard urine culture protocol to detect microorganisms. The standard protocol has been refined to grow certain uropathogens, especially *E. coli*. However,

Key advances

- Most adult women have a detectable community of bacteria in their urine[1]
- The characteristics of the female urinary microbiota relate to certain common lower urinary tract conditions, notably urgency urinary incontinence[8]
- The standard urine culture is considerably limited in detection of organisms that make up the female urinary microbiota[5]
- Enhanced quantitative urine culture techniques confirm that the DNA of the organisms detected by sequencing technology are living and cultivatable[5]
- The female urinary microbiota seems to be associated with probability of successful treatment in certain women with urgency urinary incontinence[9]

standard urine culture conditions are not ideal for many other known human uropathogens. Hilt and co-workers[2] had previously described enhanced urine culture techniques that were established to grow organisms detected previously by sequencing. In 2016, Price *et al.*[5] extended this work to a clinically relevant population of women based on their self-reported UTI. A key message of this paper was the low rate of uropathogen detection using standard urine culture techniques. Using baseline catheterized samples from 150 adults attending urogynaecology clinics, standard urine culture failed to detect 67% of uropathogens overall and 50% in participants with severe urinary symptoms. The investigators evaluated a variety of culture conditions to determine the optimal urine culture method that could be incorporated into any clinical laboratory for optimizing the detection of uropathogens. They reported that 100 μL of urine plated onto Blood (BAP), Colistin Naladixic Acid (CNA), and MacConkey agars in 5% CO_2 for 48 h resulted in detection of 84% of all uropathogens, versus just 33% with the commonly used standard urine protocol (FIG 1).

Clinicians face a number of challenges when caring for women with lower urinary tract symptoms. The simple dichotomy of 'infection' or 'no infection' does not incorporate evidence documenting the existence of the female urinary microbiota. The spectrum of health, dysbiosis, and disease likely relates to the urinary microbial community, as it does to other human microbial niches. Reliance on standard urine culture alone might limit the information available to physicians; the additional information available from enhanced urine culture techniques helps inform clinicians of the status of the urinary microbiota, including the presence of uropathogens that might be missed using standard urine culture protocols. The robust information generated by enhanced urine culture techniques provide an opportunity to advance clinical care and refine best practices in antibiotic stewardship and avoid use of interventions that wipe out bacterial communities that have a favourable biological function.

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Author contributions
Both authors researched data for article, made substantial contributions to discussions of content, wrote the article, and reviewed and edited the manuscript before submission.

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BLADDER CANCER IN 2016

News in diagnosis, treatment, and risk group assessment

Richard Zigeuner

2016 has been a good year for research in non-muscle-invasive bladder cancer. Topics to see progress include risk assessment of patients treated with BCG maintenance, the role of repeat transurethral resection of the bladder (TURB), a prospective study of white-light versus narrow-band imaging, a meta-analysis regarding single instillation chemotherapy, and the effect of the use of fluorescence-guided TURB on progression.

Many publications regarding different aspects of non-muscle-invasive bladder cancer (NMIBC) have been published over the past year, covering subjects as diverse as risk assessment, endoscopic techniques, surgery, and topical chemotherapy. An article by Cambier *et al.*[1] defined risk groups regarding recurrence, progression, cancer-specific survival, and overall survival in patients with NMIBC treated with transurethral resection of the bladder (TURB) and subsequent BCG maintenance therapy. The authors evaluated 1,812 patients from two European Organisation for Research and Treatment of Cancer (EORTC) trials and were able to stratify them into six risk groups for early recurrence based on grade, recurrence rate, and number of tumours. For late recurrence, only prior recurrence rate and number of tumours had independent effects, resulting in the creation of four risk groups. By contrast, risks of progression and death from bladder cancer were dependent only on pT classification and grade — that is, risk factors for progression from non-muscle-invasive cancer to muscle-invasive disease are different from risk factors for recurrence. The highest risk was shown for T1G3 tumours with a progression rate of nearly 20% and death from bladder cancer in 11% of patients after 5 years. The authors concluded that NMIBC is a heterogenous disease and that patients with T1G3 tumours need alternative treatments.

This study is of particular clinical importance, because the widely used EORTC risk tables[2] for prediction of bladder cancer recurrence and progression included patients

without BCG maintenance and are, therefore, not an adequate assessment tool for this population. Predictive accuracies were moderate with c-indices from 0.56 to 0.67 for recurrence and — slightly higher — from 0.64 to 0.72 for progression and death from bladder cancer. However, this study enables a risk assessment in patients treated with BCG maintenance. The conclusion that patients with T1G3 tumours are undertreated by BCG is at least debatable against the background of a cancer-specific survival (CSS) of 89% at 5 years, which seems to be not inferior to results from large series favouring early cystectomy in these patients[3].

An article by Gontero *et al.*[4] retrospectively evaluated the role of reTURB in high-grade T1G3 tumours in a multi-institutional database of 2,451 patients who were subsequently treated with BCG, 41% of whom underwent reTURB. The study end points — recurrence, progression, CSS, and overall survival— were all related to presence of detrusor muscle in the specimen after first TURB. Four groups were defined (+ or – muscle, + or – reTURB). After multivariable analysis, only patients without presence of muscle in first TURB specimen had a moderate (but not significant) outcome benefit. The current guidelines of the European Association of Urology (EAU) recommend a reTURB in all T1 and all high-grade tumours (except isolated carcinoma *in situ*), when the biopsy sample is lacking muscle in the specimen, or on the surgeon's impression of incomplete resection[5]. The 2016 article provides several surprising results. Firstly, the rate of residual tumours in the reTURB group was 71%, which is higher than average reported in the literature of 33–55%[5–7]. Secondly, only 38% of patients received maintenance BCG; as guidelines recommend BCG maintenance as standard of care, 100% of patients should ideally receive this treatment. Thirdly, no data regarding upstaging to muscle-invasive bladder cancer after reTURB are available. This omission is surprising, as after diagnosis of a T1 tumour at initial TURB, ~20% of patients are upstaged to muscle-invasive disease after reTURB. As the authors had access to the pathology reports after reTURB, data regarding upstaging should have been easily available. Fourthly, no subset of patients benefiting from reTURB could be identified, despite the considerable heterogeneity in underutilization of reTURB and BCG administration and a high rate of residual tumours. More astonishingly, recurrence rates, progression rates, and cancer-specific mortality were 51%, 19%, and 9%, respectively, after a median follow-up period of more than 5 years. These outcomes are much better than expected when looking at the presented residual tumour rates and, regarding CSS, seem to be noninferior even to early cystectomy series for T1G3 (REF. 3). Possible reasons for these unexpected results include the fact that potential harms of inadequate TURB might be compensated by ablative effects of BCG. Alternatively, perhaps a salvage TURB at 3 months for so-called early recurrence provides the same outcomes as a reTURB at 4–6 weeks. These reasons could only be properly investigated in a prospective randomized trial, which is not currently in sight.

Naito *et al.*[8] published the results of a prospective randomized trial comparing white-light (WL)–TURB with TURB using narrow-band imaging (NBI), with the primary end point of recurrence rate at 1 year after treatment. The study hypothesis was a reduction of recurrence by NBI of 10%. 981 patients were randomized and stratified by EORTC risk group (low, intermediate, high). The overall recurrence rates were ~16% at 3 months and 26% at 12 months. Overall, no significant reduction in recurrence rates at 3 months or 12 months could be demonstrated for NBI versus WL-TURB. In preplanned subgroup analysis, only patients with low-risk tumours showed an absolute risk reduction of 15% and 22% at 3 months and 12 months, respectively. The percentage of risk reduction seems to be clinically relevant; however, the low-risk group represented the smallest sample size in this study, and by absolute numbers recurrences could be prevented by the use of NBI in eight (at 3 months) and 12 (at 12 months) patients, whereas the larger subsets of patients with intermediate-risk and high-risk had no benefit. Moreover, the dropout rate in this study was >40%. The main limitation of this study is the lack of information regarding adjuvant intravesical treatment. In summary, the benefit of NBI seems to be very limited, as only a small number of patients with low-risk disease saw a benefit.

> "…potential harms of inadequate TURB might be compensated by ablative effects of BCG"

The topic of single instillation chemotherapy after TURB versus TURB alone was evaluated by Sylvester *et al.*[9] in an individual patient data meta-analysis of 11 randomized studies including 2,278 patients. Single instillation chemotherapy reduced the risk of recurrence by 35% (HR 0.65), the absolute risk reduction was 14% (from 59% to 45%) at 5 years, corresponding to a number needed to treat (NNT) of seven instillations to prevent one recurrence. No benefit was demonstrated in patients with a prior recurrence rate of >1 per year and with an EORTC recurrence score of ≥5. No benefit in time to progression or death from bladder cancer was observed; however, in patients with EORTC score ≥5, increased overall mortality was noted (HR 1.26), of 12% in those with EORTC score ≥5 versus 11.2% in those with EORTC score <5. The authors concluded that single-instillation chemotherapy should not be given in patients with high-risk disease. Sylvester and colleagues' study provides clear data regarding the reduction in cancer recurrence risk by single instillation chemotherapy. The finding that patients with a recurrence risk score of ≥5 do not benefit seems somewhat academic at a first glance — as the EORTC tables[2] combine clinical and pathological parameters, the latter are not available at time of instillation. However, any multifocal recurrent tumour (and this information is available at time of surgery) has a score of ≥5 and could be excluded from single instillation. The reason for an increased overall mortality in these patients after single instillation remains

Key advances

- Data from EORTC studies were used to define risk groups of patients with NMIBC treated with BCG maintenance regarding recurrence, progression, and cancer-specific mortality[1]
- A retrospective multi-institutional analysis evaluating the role of reTURB in T1G3 patients subsequently treated with BCG showed a high rate of residual cancer and only limited clinical benefits[4]
- TURB using narrow-band imaging versus white light reduced recurrence rates only in a very small number of patients with low risk tumours, whereas the overall study population did not benefit[8]
- Single instillation chemotherapy after TURB reduced the absolute recurrence risk by 14% in a meta-analysis, but only in patients with EORTC risk scores <5 and/or <1 recurrence per year[9]
- For the first time, fluorescence-guided TURB was shown to reduce the risk of progression by 4% compared with white light in a meta-analysis[10]

unclear, and the largest subgroup were affected by malignancies other than bladder cancer. The Kaplan–Meier curves for overall mortality of subgroups of patients with an EORTC score ≥5 who either did or did not receive single instillation chemotherapy separate after 6 years, and, in absolute numbers, the difference is 21 patients. However, the difference might have occurred by chance, as this study was not designed for a preplanned subgroup analysis.

Finally, Gakis and Fahmy[10] reported a meta-analysis regarding the effect of fluorescence-guided TURB using hexaminolevulinate (HAL) versus WL on progression. Five studies (four prospective, one retrospective) including 1,301 patients were selected. After a median follow-up period of 27.6 months (HAL) versus 29 months (WL), progression was noted in 6.8% of patients who underwent HAL imaging and 10.7% of those who received WL imaging (OR = 1.64, $P = 0.01$). The absolute difference in progression rate is close to 4%, corresponding to a NNT of 25 HAL-guided TURBs to prevent one progression. This meta-analysis shows for the first time not only an increased detection of bladder lesions by HAL but also a reduction in progression risk. The authors hypothesize that this effect might be the result of an increased detection and eradication of carcinoma *in situ* by HAL. However, one limitation of the study is the heterogenous definition of progression between the five trials. In my opinion, it seems plausible that improved eradication of flat tumour lesions including carcinoma *in situ*, which is usually not visible with white light but can be detected by HAL and has a high potential to progress to muscle-invasive disease if left untreated, is the main reason for this effect.

Overall in 2016: updated EORTC risk groups provide outcome assessment for BCG-treated patients, reTURB in patients with T1G3 bladder cancer showed a high residual tumour rate and very moderate benefits in a real life setting, and NBI reduced early recurrence rates compared with white light, but only in low-risk tumours in a small number of patients. A single instillation chemotherapy after TURB was effective with respect to reduced risk of recurrence in patients with EORTC risk scores <5 or prior recurrence rates of <1 per year, whereas fluorescence-guided TURB was shown to reduce risk of progression for the first time in a meta-analysis.

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KIDNEY CANCER IN 2016

RCC — advances in targeted therapeutics and genomics

W. Marston Linehan and Christopher J. Ricketts

Recent advances have been exciting in the genomics of and targeted therapeutics for renal cell carcinoma (RCC). New agents have been approved for advanced RCC, a novel agent targeting hypoxia-inducible factor 2α has shown considerable promise and molecular characterization of papillary RCC provides the foundation for development of targeted therapeutic approaches for this disease.

Renal cell carcinoma (RCC) affects nearly 300,000 individuals worldwide annually and is responsible for over 100,000 deaths each year. RCC is not a single entity, but is made up of several different types of cancer that present with different histologies, clinical courses, and responses to therapy, which are associated with different genetic alterations. Clear cell RCC (ccRCC) is the most common type (~75%) and the most well-characterized form of RCC. ccRCC is genetically distinguished by inactivation of *VHL*, most commonly by mutation or methylation of *VHL* and loss of chromosome 3p. The protein product of *VHL*, pVHL, is a component of an E3 ubiquitin ligase complex that targets the HIFs, HIF1α and HIF2α, for ubiquitin-mediated degradation in an oxygen-sensitive manner. When *VHL* is lost in ccRCC, even in normoxia, the VHL complex no longer targets and degrades the HIF transcription factors, and HIF accumulates and activates the hypoxia response pathway. Activation of the hypoxia response pathway results in increased expression of HIF target genes, such as *VEGF*, *PDGF*, and *TGFA*, which can promote angiogenesis, proliferation, and migration that aid tumorigenesis[1]. Elucidation of the VHL pathway has provided the foundation for the development of targeted therapeutic agents for patients with advanced RCC.

From 2005–2015, seven new agents targeting the VHL–HIF pathway were approved by the FDA for the treatment of patients with advanced RCC. Five of these agents, sorafenib, sunitinib, bevacizumab, pazopanib, and axitinib, directly target downstream targets of the HIF pathway, such as VEGF, VEGFR, and PDGFR; whereas, two agents, temsirolimus and everolimus, inhibit the mTOR pathway and induce reduced levels of HIF by inhibiting *de novo* translation and

Key advances

- Two novel agents, cabozantinib and lenvatinib, have been approved for the treatment of patients with advanced renal cell carcinoma (RCC)
- Sunitinib has been shown to significantly improve the median duration of disease-free, post-surgical survival in patients with locoregional clear cell (cc) RCC with a high risk of tumour recurrence
- Seminal studies demonstrate the ability to directly target HIF2α transcription with small-molecule inhibitors and that targeting HIF2α in VHL-deficient ccRCCs can have a substantial antitumour effect
- The Cancer Genome Atlas Research Network reported the comprehensive molecular characterization of papillary (p) RCC, confirming the distinctiveness of type 1 and type 2 pRCC, and characterizing the heterogeneity of type 2

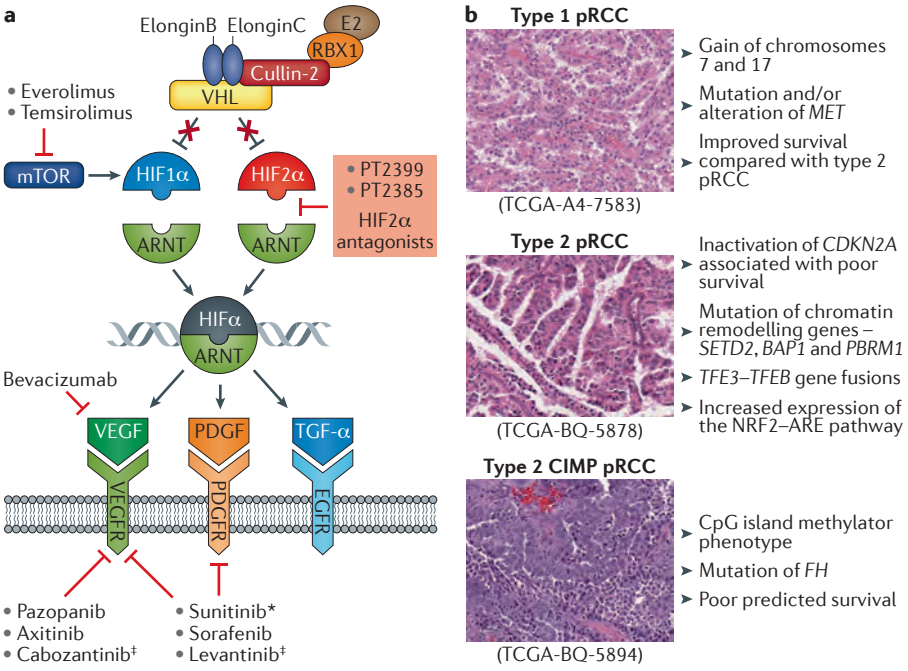

Figure 1 | **Recent advances in treatment and characterization of renal cell carcinoma.** **a** | The VHL complex (including elongin B and elongin C, cullin 2, RBX1, and an E2 ubiquitin-conjugating enzyme) degrades HIF1α and HIF2α in normoxia. VHL loss in clear cell (cc)RCC stabilizes the HIFs, enabling dimerization with ARNT and activation of downstream targets of the hypoxia response pathway, such as VEGF, PDGF, and TGFα. Therapeutic agents for advanced ccRCC can either target the activated components of the hypoxia response pathway (bevacizumab, pazopanib, axitinib, cabozantinib, sunitinib, sorafenib, and levantinib), such as VEGF, VEGFR, and PDGFR, or target the mTOR pathway (temsirolimus and everolimus) to decrease levels of HIF by inhibiting translation of de novo HIF protein. The novel therapeutic antagonists PT2399 and PT2385 for treating advanced ccRCC specifically inhibit the dimerization of HIF2α and ARNT, resulting in loss of HIF2α downstream target gene expression. *Sunitinib improves the median duration of disease-free postsurgical survival in patients with localized, high-recurrence-risk ccRCC. ‡Cabozantinib and a combination of levantinib and everolimus have been approved as second-line therapies for patients with ccRCC who have progressed on alternative VEGFR inhibitors. **b** | The Cancer Genome Atlas Research Network analysis of papillary (p)RCC subclassified the tumours into type 1 and type 2 pRCC based on histology and characterized the genetic alterations associated with each type. Included within type 2 pRCC is a subset of tumours characterized by a CpG island methylator phenotype (CIMP) and associated with FH mutation and significantly shorter patient survival.

are used as both first-line and second-line therapies (FIG. 1a).

In 2016, two novel agents, cabozantinib and lenvatinib, were approved for the treatment of patients with advanced RCC. Cabozantinib is a multiple tyrosine kinase inhibitor (TKI) that targets VEGFR, as well as MET and AXL. A randomized phase III trial evaluated the effect of cabozantinib as a second-line therapy in 658 patients with advanced ccRCC who had progressed after treatment with one or more VEGFR TKIs[2]. Patients who received cabozantinib had an improved median overall survival (OS) of 21.4 months compared with 16.5 months for those who received everolimus (hazard ratio (HR) 0.66, 95% CI 0.53–0.83; $P = 0.00026$), an improved progression-free survival (PFS) (HR 0.51, 95% CI 0.41–0.62; $P < 0.0001$), and an increased response rate in comparison with everolimus (17% versus 3%; $P < 0.0001$) (FIG. 1a)[2]. Lenvatinib is a TKI that also targets VEGFRs, as well as FGFR1–FGFR4, PDGFR, RET and KIT. A phase II registration study was performed to evaluate the therapeutic effect of lenvatinib as a second-line therapy in 153 patients with advanced ccRCC who had progressed after a previous VEGF-targeted therapy[3] The everolimus and lenvatinib combination treatment improved median PFS to 14.6 months compared with 5.5 months in patients treated with single-agent everolimus (HR 0.40, 95% CI 0.24–0.68; $P = 0.0005$), improved median OS to 25.5 months compared with 15.4 months (HR 0.51, 95% CI 0.30–0.88; $P = 0.024$), and significantly increased objective response rate (43% versus 6%; $P < 0.0001$)[3]. The FDA approved cabozantinib in April 2016 and the combination of lenvatinib and everolimus in May 2016 as second-line therapies for patients with advanced ccRCC. In addition, in October 2016, sunitinib was shown to significantly improve the median duration of disease-free postsurgical survival from 5.6 years with placebo to 6.8 years in patients with locoregional ccRCC with a high risk of tumour recurrence (HR 0.76, 95% CI 0.59–0.98; $P = 0.03$) (FIG. 1a)[4].

The ability to target the VHL–HIF2α pathway in VHL-deficient ccRCC has long been a goal of investigators in this field. Two recent studies have evaluated a novel therapeutic approach that directly targets HIF2α using a small-molecule inhibitor, PT2399. PT2399 was designed to directly bind the PAS B domain of HIF2α and inhibit binding to its dimerization partner, ARNT, which is essential for HIF2α transcriptional activity[5,6]. Cho *et al.*[6] evaluated the effectiveness of PT2399 in both *in vitro* and *in vivo* cell-line models of VHL-deficient ccRCC and demonstrated inhibited colony formation and tumour regression in mouse xenograft models[6]. Chen *et al.*[5] evaluated tumorigenesis in a human ccRCC tumourgraft–patient-derived-xenograft (PDX) platform and showed suppressed tumorigenesis in 56% (10/18) of the PDXs treated with PT2399 (REF. 5). Both studies demonstrated the on-target effects of the HIF2α inhibition. The sensitive PDX models had robust HIF2α protein expression and a specific mRNA expression signature associated with response was identified by comparison with the resistant PDX model[5,6]. These seminal studies clearly demonstrate the ability to directly target HIF2α transcription and that targeting HIF2α in VHL-deficient

ccRCCs can have a substantial antitumour effect. An eagerly awaited clinical trial of a companion agent, PT2385, is currently underway and has the potential for the development of a whole new class of agents targeting the VHL–HIF2α pathway for patients with VHL-deficient ccRCC (FIG. 1a).

> “…analysis confirmed the distinctiveness of type 1 and type 2 pRCC…”

The Cancer Genome Atlas (TCGA) Research Network has previously conducted comprehensive molecular characterization of both ccRCC and chromophobe (ch)RCC[7,8]. Recently, TCGA Research Network reported the comprehensive molecular characterization of papillary (p)RCC[9]. This analysis confirmed the distinctiveness of type 1 and type 2 pRCC, and characterized the heterogeneity of type 2 pRCC (FIG. 1b). Type 1 pRCC was shown to be characterized by a clear pattern of copy number gains for chromosomes 7 and 17, an association with *MET* mutation or alteration, and an improved survival rate compared with type 2 pRCC. Type 2 pRCC was shown to have a variety of genetic alterations, which included loss of *CDKN2A* (associated with decreased survival), and mutation of chromatin remodelling genes including *BAP1*, *SETD2*, and *PBRM1*. Furthermore, *TFE3–TFEB* gene fusions, which are usually thought to be associated with childhood RCC[10], were observed in a relatively high percentage (12%) of type 2 pRCCs from adults. Type 2 pRCCs were also shown to have increased expression of the NRF2–ARE pathway genes and mutations in the genes that regulate the NRF2–ARE pathway were identified in a subset of type 2 pRCCs. Notably, a subset of type 2 pRCC samples demonstrated a distinct CpG island methylator phenotype (CIMP) that was associated with germline or somatic mutation of *FH*, highly increased expression of the NRF2–ARE pathway, and a significantly decreased survival ($P<0.0001$) (FIG. 1b)[9]. These findings provide the foundation for understanding the molecular basis of pRCC which will hopefully result in the development of effective therapies for this disease.

In summary, new second-line therapies for patients with advanced ccRCC have recently been approved and a novel therapeutic approach targeting of HIF2α transcription and improved understanding of the molecular basis of pRCC will hopefully result in improved management approaches for patients affected with this disease.

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Competing interests statement
The authors declare no competing interests.

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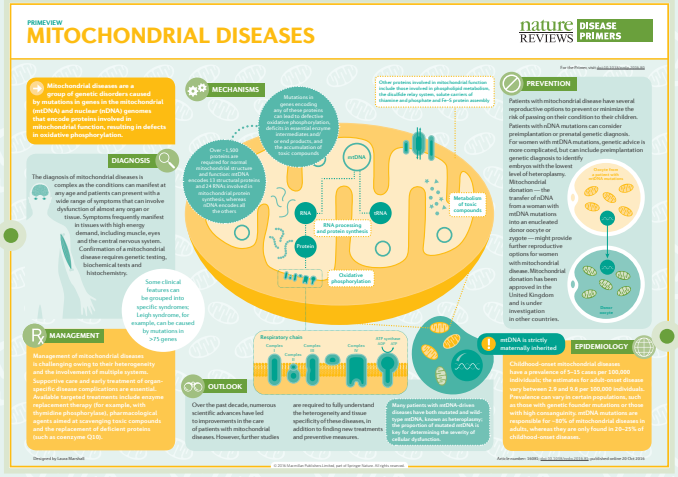
nature REVIEWS DISEASE PRIMERS

Mitochondrial diseases

Mitochondrial diseases are a group of genetic disorders characterized by mutations in nuclear or mitochondrial DNA, causing a range of manifestations that can present at various times.

Animation

The animation describes the genetic defects involved and explores the options available to stop inheritance of faulty maternal mitochondrial DNA.



Primer article

Gráinne S. Gorman, Patrick F. Chinnery, Salvatore DiMauro, Michio Hirano, Yasutoshi Koga, Robert McFarland, Anu Suomalainen, David R. Thorburn, Massimo Zeviani & Douglass M. Turnbull

The Primer discusses the mechanisms underlying the development of mitochondrial diseases, in addition to the diagnosis, prevention and management of these disorders.

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