5 Research Breakthroughs in The Past Year

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Disclosures

• I am a consultant with:
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Challenges in the Current Era of MS Treatment

- **Diagnostic accuracy & precision**
- **Ability to prognosticate**
  - Ways to predict individual patient long-term disability outcomes to guide therapy choices
  - Better ways to quickly assess treatment response to DMTs
- **Treatment of progressive MS**
  - ~ 50% of RRMS goes on to SPMS without intervention
  - PPMS, PRMS: ~ 10-15% of MS patients
- **Neuro-repair**
Utility of Biomedical Research

Discovery

Clinical Application
Breakthrough #1

B cells
Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis

Rituxan – patent expired in 2015
Ocrelizumab – patent

Phase I: Researchers test a new drug or treatment in a small group of people for the first time to evaluate its safety, determine a safe dosage range, and identify side effects.

Phase II: The drug or treatment is given to a larger group of people to see if it is effective and to further evaluate its safety.

Phase III: The drug or treatment is given to large groups of people to confirm its effectiveness, monitor side effects, compare it to commonly used treatments, and collect information that will allow the drug or treatment to be used safely.
Ocrelizumab versus Placebo in Primary Progressive Multiple Sclerosis

Hazard ratio, 0.75 (95% CI, 0.58–0.98)
P = 0.04

No. at Risk
Placebo: 244, 234, 214, 202, 193, 183, 176, 166, 157, 148, 139, 125, 89, 70, 50, 33, 22, 7, 2
Ocrelizumab: 487, 465, 454, 437, 421, 397, 384, 367, 349, 330, 313, 290, 217, 177, 144, 87, 50, 21, 7

Future Directions

• Determine how B cells contribute to MS pathogenesis (RRMS & PMS)
• Characterize and reduce potential side-effects from B cell depletion therapy
• Clarifying role of additional B cell depletion therapeutics
Breakthrough #2

Imaging
Lymphatic System

Structural and functional features of central nervous system lymphatic vessels

Antoine Louveau\textsuperscript{1,2}, Igor Smirnov\textsuperscript{1,2}, Timothy J. Keyes\textsuperscript{1,2}, Jacob D. Eccles\textsuperscript{3,4,5}, Sherin J. Rouhani\textsuperscript{3,4,6}, J. David Peske\textsuperscript{3,4,6}, Noel C. Derecki\textsuperscript{1,2}, David Castle\textsuperscript{7}, James W. Mandell\textsuperscript{8}, Kevin S. Lee\textsuperscript{1,2,9}, Tajie H. Harris\textsuperscript{1,2} & Jonathan Kipnis\textsuperscript{1,2,3}
Human and nonhuman primate meninges harbor lymphatic vessels that can be visualized noninvasively by MRI

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Abstract Here, we report the existence of meningeal lymphatic vessels in human and nonhuman primates (common marmoset monkeys) and the feasibility of noninvasively imaging and mapping them in vivo with high-resolution, clinical MRI. On T2-FLAIR and T1-weighted black-blood imaging, lymphatic vessels enhance with gadobutrol, a gadolinium-based contrast agent with high propensity to extravasate across a permeable capillary endothelial barrier, but not with gadofosveset, a blood-pool contrast agent. The topography of these vessels, running alongside dural venous sinuses, recapitulates the meningeal lymphatic system of rodents. In primates, meningeal lymphatics display a typical panel of lymphatic endothelial markers by immunohistochemistry. This discovery holds promise for better understanding the normal physiology of lymphatic drainage from the central nervous system and potential aberrations in neurological diseases.

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Figure 2. Gadobutrol vs. gadofosveset in MRI-visualization of dural lymphatic vessels. Coronal T1-weighted black-blood images were acquired after intravenous administration of either Gadobutrol or Gadofosveset.
Future Directions

• Identification of human immune cell trafficking \textit{in vivo}
• Dynamic relation between lymphatics and B cells in CNS
• Capturing features of CNS ectopic lymphoid follicles in MS
• Features of meningeal space after MS therapy
Breakthrough #3

The Microbiome
Gut microbiota from multiple sclerosis patients enables spontaneous encephalomyelitis in mice

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There is emerging evidence that the commensal microbiota has a role in the pathogenesis of multiple sclerosis (MS), a putative autoimmune disease of the CNS. Here, we compared the gut microbial composition of 34 monozygotic twin pairs discordant for MS. While there were no major differences in the overall microbial profiles, we found a significant increase in some taxa such as Akkermansia in untreated MS twins. Furthermore, most notably, when transplanted to a transgenic mouse model of spontaneous brain autoimmune, MS twin-derived microbiota induced a significantly higher incidence of autoimmunity than the healthy twin-derived microbiota. The microbial profiles of the colonized mice showed a high intraindividual and remarkable temporal stability with several differences, including Sutterella, an organism shown to induce a protective immunoregulatory profile in vitro. Immune cells from mouse recipients of MS-twin samples produced less IL-10 than immune cells from mice colonized with healthy-twin samples. IL-10 may have a regulatory role in spontaneous CNS autoimmunity, as neutralization of the cytokine in mice colonized with healthy-twin fecal samples increased disease incidence. These findings provide evidence that MS-derived microbiota contain factors that precipitate an MS-like autoimmune disease in a transgenic mouse model. They hence encourage the detailed search for protective and pathogenic microbial components in human MS.

Results

MZ Twin Cohorts Discordant for MS. We assembled a cohort of 34 MZ twin pairs clinically discordant for MS. In each pair, one twin has clinically definite MS according to the current diagnostic criteria (11), whereas the co-twin is unaffected. Our MS twin cohort resembles the general MS population with respect to female

Significance
Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models

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The gut microbiota regulates T cell functions throughout the body. We hypothesized that intestinal bacteria impact the pathogenesis of multiple sclerosis (MS), an autoimmune disorder of the CNS and thus analyzed the microbiomes of 71 MS patients not undergoing treatment and 71 healthy controls. Although no major shifts in microbial community structure were found, we identified specific bacterial taxa that were significantly associated with MS. Akkermansia muciniphila and Acinetobacter calcoaceticus, both increased in MS patients, induced proinflammatory responses in human peripheral blood mononuclear cells and in monocolonized mice. In contrast, Parabacteroides distasonis, which was reduced in MS patients, stimulated antiinflammatory IL-10–expressing human CD4\textsuperscript{+}CD25\textsuperscript{+} T cells and IL-10–FoxP3\textsuperscript{+} Tregs in mice. Finally, microbiota transplants from MS patients into germ-free mice resulted in more severe symptoms of experimental autoimmune encephalomyelitis and reduced proportions of IL-10\textsuperscript{+} Tregs compared with mice “humanized” with microbiota from healthy controls. This study identifies specific human gut bacteria that regulate adaptive autoimmune responses, suggesting therapeutic targeting of the microbiota as a treatment for MS.

Results

The MS Microbiome Elicits Differential Treg Responses and Shows Modest Dysbiosis at the Genus Level. To investigate whether MS-associated bacteria affect immune functions in the host, we stimulated peripheral blood mononuclear cells (PBMCs) from MS patients or healthy controls, using extracts from total bacteria isolated from the stool samples of the same subjects who were PBMC donors (thus, “self” bacterial extracts). We observed that PBMCs from MS patients showed an impaired ability to differentiate or expand CD25\textsuperscript{+}FoxP3\textsuperscript{+} Treg populations (Fig. 1A). The total CD3\textsuperscript{+}CD4\textsuperscript{+} Th lymphocyte population was not altered by bacterial extract treatment, and the baseline proportion of CD25\textsuperscript{+}FoxP3\textsuperscript{+} Tregs (in a population of CD3\textsuperscript{+}CD4\textsuperscript{+} T cells) was not different between MS patients and healthy controls. These results suggest a specific immunoregulatory role of microbiota on PBMCs from MS patients.

We subsequently analyzed the microbiome by 16S rRNA gene sequencing of stool samples from 71 untreated relapsing–remitting
Cell Metabolism

Intermittent Fasting Confers Protection in CNS Autoimmunity by Altering the Gut Microbiota

Graphical Abstract

Highlights
- IF ameliorates the clinical course and pathology of the MS mouse model (EAE)
- IF increases gut microbial diversity, alters their composition and metabolic pathways
- Gut microbiota transfer from mice on IF led to protection from EAE in recipient mice
- Findings with IER in MS patients partially recapitulates what is observed with IF in EAE

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In Brief
Intermittent fasting confers protection in the multiple sclerosis animal model through effects on the gut microbiota; similar changes to the gut microbiota were observed in relapsing multiple sclerosis patients undergoing intermittent energy restriction.
Future Directions

• Identification of ‘pathogenic’ microbiota
• Determine optimal manipulation of gut flora
• Clarify mechanisms of microbiotal influences on immune and nervous systems
• Commonalities and uniqueness amongst autoimmune diseases
Breakthrough #4

Immune targets
Gsk3 is a metabolic checkpoint regulator in B cells

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B cells predominate in a quiescent state until an antigen is encountered, which results in rapid growth, proliferation and differentiation of the B cells. These distinct cell states are probably accompanied by differing metabolic needs, yet little is known about the metabolic control of B cell fate. Here we show that glycogen synthase kinase 3 (Gsk3) is a metabolic sensor that promotes the survival of naive recirculating B cells by restricting cell mass accumulation. In antigen-driven responses, Gsk3 was selectively required for regulation of B cell size, mitochondrial biogenesis, glycolysis and production of reactive oxygen species (ROS), in a manner mediated by the co-stimulatory receptor CD40. Gsk3 was required to prevent metabolic collapse and ROS-induced apoptosis after glucose became limiting, functioning in part by repressing growth dependent on the myelocytomatosis oncoprotein c-Myc. Notably, we found that Gsk3 was required for the generation and maintenance of germinal center B cells, which require high glycolytic activity to support growth and proliferation in a hypoxic microenvironment.
Dimethyl fumarate targets GAPDH and aerobic glycolysis to modulate immunity

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Activated immune cells undergo a metabolic switch to aerobic glycolysis akin to the Warburg effect, presenting a potential therapeutic target in autoimmune disease. Dimethyl fumarate, a derivative of the Krebs cycle intermediate fumarate, is an immunomodulatory drug used to treat multiple sclerosis and psoriasis. Although its therapeutic mechanism remains uncertain, it covalently modifies cysteine residues in a process termed “succination.” Here, we show that dimethyl fumarate succinates and inactivates the catalytic cysteine of the glycolytic enzyme GAPDH both in vitro and in vivo. It thereby downregulates aerobic glycolysis in activated myeloid and lymphoid cells, which mediates its anti-inflammatory effects. Our findings provide mechanistic insight into immune modulation by dimethyl fumarate and represent a proof of concept that aerobic glycolysis is a therapeutic target in autoimmunity.
Future Directions

• Define impact of immuno-metabolism on the pathogenesis of MS
• Identify whether additional disease-modifying therapies target metabolic pathways
• Identify and apply selective immuno-metabolism targeting agents in MS
Breakthrough #5

Outside the Box?
Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy


ABSTRACT

BACKGROUND
Spinal muscular atrophy is an autosomal recessive neuromuscular disorder that is caused by an insufficient level of survival motor neuron (SMN) protein. Nusinersen is an antisense oligonucleotide drug that modifies pre–messenger RNA splicing of the SMN2 gene and thus promotes increased production of full-length SMN protein.

METHODS
We conducted a randomized, double-blind, sham-controlled, phase 3 efficacy and safety trial of nusinersen in infants with spinal muscular atrophy. The primary end points were a motor-milestone response (defined according to results on the Hammersmith Infant Neurological Examination) and event-free survival (time to death or the use of permanent assisted ventilation). Secondary end points included overall survival and subgroup analyses of event-free survival according to disease duration at screening. Only the first primary end point was tested in a prespecified interim analysis. To control the overall type I error rate at 0.05, a hierarchical testing strategy was used for the second primary end point and the secondary end points in the final analysis.

The authors’ full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Finkel at the Division of Neurology, Department of Pediatrics, Nemours Children’s Hospital, 13535 Nemours Pkwy., 5th Fl., Orlando, FL 32827, or at richard.finkel@nemours.org.

*A complete list of the principal investigators in the ENDEAR trial is provided in the Supplementary Appendix, available at NEJM.org.

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Figure 2. Event-free Survival and Overall Survival.

Panel A shows the probability of event-free survival (the proportion of infants who were alive without the use of permanent assisted ventilation) and Panel B shows the probability of overall survival (the proportion of infants who were alive) in the nusinersen group and the control group. The median time to death or the use of permanent assisted ventilation was 22.6 weeks in the control group and was not reached in the nusinersen group; the median time to death was not reached in either group.
Future Directions

• Identify optimal targets for neuroprotection
• Identify optimal timing for targeting neurons in MS
• Characterize utility of neuroprotective therapies in the context of current disease-modifying therapies
Utility of Biomedical Research

Discovery → Clinical Application
Utility of Biomedical Research

Discovery

Refinement of Question

Clinical Application