T cells. It is FOXP3 that programmes $T_{reg}$ cell development and function, as revealed by the findings that ectopic FOXP3 expression conferred a $T_{reg}$ cell-like phenotype and function on conventional T cells and that loss-of-function *Foxp3* mutations led to defective development of functional $T_{reg}$ cells. $T_{reg}$ cells are indeed indispensable for immune tolerance, because the $T_{reg}$ cell deficiency was shown to be the primary cause of the fatal autoimmune disease in *scurfy* mice.

> at the dawn of the twenty-first century, there was still deep-rooted scepticism about $T_{reg}$ cells

These findings led to the notion that FOXP3 functions as a 'master regulator' of $T_{reg}$ cells. In retrospect, this was actually an oversimplified view in that we now know that FOXP3 functions in a chromatin landscape that is established in a FOXP3-independent manner and cooperates with other transcription factors to control multiple facets of $T_{reg}$ cell differentiation and function. Nevertheless, these three papers transformed immunology and biomedical sciences by providing a molecular key to address the diverse and fundamental immunological and non-immunological functions of $T_{reg}$ cells in health and disease and to elucidate the molecular and cellular mechanisms of $T_{reg}$ cell differentiation and function. Like many important scientific discoveries, these studies have opened new directions of research, inspired numerous fruitful findings and will continue to do so in the future.

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**ORIGINAL ARTICLES** Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y. Foxp3 programs the development and function of CD4$^+$CD25$^+$ regulatory T cells. *Nat. Immunol.* **4**, 330–336 (2003) | Hori, S., Nomura, T. & Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**, 1057–1061 (2003) | Khattri, R. et al. An essential role for Scurfin in CD4$^+$CD25$^+$ T regulatory cells. *Nat. Immunol.* **4**, 337–342 (2003)
**RELATED ARTICLE** Ramsdell, F. & Ziegler, S. F. FOXP3 and scurfy: how it all began. *Nat. Rev. Immunol.* **14**, 343–349 (2014)

generation and immunity to cancer (Pearce et al., 2009). Furthermore, MYC was shown to control metabolic reprogramming in activated T cells, highlighting that transcriptional remodelling underlies the robustness of metabolic changes after activation (Wang et al., 2011). Other important work showed that distinct CD4$^+$ T cell subsets use different metabolic pathways to support their function, with T helper 1 ($T_H1$), $T_H2$ and $T_H17$ cells engaging glycolysis and regulatory T cells preferring fatty acid oxidation (Michalek et al., 2011). In parallel, the importance of cell-intrinsic changes in metabolism for innate immunity became apparent when Toll-like receptor agonists were shown to stimulate a metabolic transition to glycolysis in dendritic cells, with activation-associated functions again linked to metabolic state (Krawczyk et al., 2010). These reports laid the groundwork for the field of 'immunometabolism', which has grown explosively in the past decade.

The ever-growing literature on immunometabolism has described exciting and unanticipated new aspects of metabolic pathways, and of metabolite biology, that fundamentally impact cellular function and immunity. The great potential here is that facets of metabolism that are preferentially emphasized in immune cells will provide targetable pathways for new therapeutic approaches to promote or inhibit immune responses to ameliorate disease.

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**Competing interests**
The author is a SAB member of ImmunoMet and a Founder of Rheos Medicines.

**ORIGINAL ARTICLES** Frauwirth, K. A. et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity* **16**, 769–777 (2002) | Pearce, E. L. et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* **460**, 103–107 (2009) | Wang, R. et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* **35**, 871–882 (2011) | Michalek, R. D. et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4$^+$ T cell subsets. *J. Immunol.* **186**, 3299–3303 (2011) | Krawczyk, C. M. et al. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* **115**, 4742–4749 (2010)

# Journal Club

NRI AT 20: RNA VACCINES

## MODIFIED URIDINES ARE THE KEY TO A SUCCESSFUL MESSAGE

In the 1990s, we started to investigate mRNA as a platform for protein replacement therapy. As these mRNAs encoded self-proteins, we did not think that mRNA transfection would generate any adverse immune effects. However, we found that transfecting human dendritic cells (DCs) with mRNA, or even with non-coding ribonucleotide homopolymers, induced inflammatory cytokines (Ni, H. et al., 2002).

At the time, we knew that DNA activates Toll-like receptor 9 (TLR9) and that double-stranded RNA can activate TLR3 and induce type I interferon. We hypothesized that one of the remaining TLR family members might sense single-stranded RNA. We also started to explore the activation of human DCs by different types of RNA to determine whether they all induce inflammatory cytokines. Natural RNAs are synthesized from the four basic nucleotides, but some of the nucleosides can be post-transcriptionally modified. We found that tRNA, which is known to be enriched in modified nucleosides, was non-inflammatory, and that TLR7 and TLR8 sense single-stranded RNA. We set out to generate RNA with modified nucleosides by in vitro synthesis. Surprisingly, the replacement of uridine with pseudouridine rendered the RNAs non-immunogenic (Karikó, K. et al., 2005).

In subsequent studies we demonstrated that mRNA containing pseudouridine was an ideal molecule for protein replacement therapy because it was efficiently translated and, unlike its unmodified counterpart, did not induce interferon in mice. Indeed, the injection of a small amount of mRNA was sufficient for the encoded protein to exert its therapeutic effect (Karikó, K. et al., 2008; Karikó, K. et al., 2012).

In parallel to these studies, we investigated mRNA as a platform for vaccine development. We predicted that uridine-containing (and thereby self-adjuvanted) mRNA encoding viral antigens would be optimal for vaccine development. Amazingly, non-immunogenic mRNA containing modified uridines also turned out to be a more suitable molecule for vaccine development (Pardi et al., 2017). Indeed, the first mRNA-based vaccines to receive regulatory authorization — developed by Moderna and by BioNTech/Pfizer for COVID-19 — are both based on 1-methylpseudouridine-containing mRNA.

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**Competing interests**
K.K. is named on patents that describe the use of nucleoside-modified mRNA as a platform to deliver therapeutic proteins. She is employee of BioNTech SE, a company that develops mRNA-based therapies.

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