for binding were identified for both peptides. This highlights the utility of the technique to analyze multiple TCR–pMHC interactions, as in the method described by Zhang *et al.*[1] Interestingly, mutations in peptide 'anchor' residues did not hinder peptide–MHC interactions as predicted, suggesting that these residues do not have a major role in TCR recognition.

In a larger experiment, the authors analyzed the cross-reactive potential of 12 TCRs from 4 patients with Merkel cell carcinoma for the same pMHC antigen by deriving all possible peptide variants, which could provide valuable information on TCR risk ($n = 192$). Each TCR could potentially cross-react with between 12 and ~28,000 peptide variants. There was an inverse correlation between the number of targets of the TCR and T-cell efficacy, as determined by interferon-γ secretion, suggesting that this technique can shed light on T cell functionality as well as cross-reactivity. This finding remains to be corroborated by analyzing more TCRs and possible pMHC binders.

The authors also studied the cross-reactivity of the 12 TCRs against the entire human proteome by synthesizing 75 predicted targets. One of the TCRs cross-reacted with a peptide derived from a protein expressed in myocytes, confirming that this method[9] could be useful to improve the safety of TCR therapies.

Both Zhang *et al.*[3] and Bentzen *et al.*[4] achieve high-throughput analysis of cross-reactivity using existing 'one-pot' methods, which allow multiple TCR–pMHC interactions to be studied simultaneously. The strengths of Zhang *et al.*[1] are the use of *in vitro* transcription and translation for faster peptide synthesis and the smart choice to use the same oligonucleotides to produce the DNA barcodes and the peptides for MHC binding, which removes bias due to differences in barcode conjugation. Variations in conjugation of DNA barcodes to the dextran polymer in the method of Bentzen *et al.*[4] could be problematic in TCR fingerprinting as this could introduce error in the antigen binding hierarchy. While Zhang *et al.*[3] use single-cell sorting to isolate TCR and peptide binder sequences, Bentzen *et al.*[4] perform sequencing on bulk T-cell populations. Adding an extra step of single-cell sorting and sequencing to the latter's protocol would also enable single-T-cell TCR fingerprinting, without any disadvantages.

Studying the molecular interactions of pMHC and TCRs by X-ray crystallography is often time consuming. Bentzen *et al.*[4] provide a relatively quick way to determine TCR fingerprints, which is useful for modeling these structures. Until now, alanine-scanning mutagenesis and individual mutant-peptide binding analysis was the most common approach to studying TCR cross-reactivity, but Bentzen *et al.*[4] substitute all possible amino acids and then characterize cross-reactivity for peptide variants in a one-pot approach. This method is also advantageous for evaluating potential cross-reactivity of clinically relevant TCRs against the entire human proteome. The neoantigen-specific TCRs identified by Zhang *et al.*[3] could be tested for cross-reactivity against the whole proteome using the method of Bentzen *et al.*[4] by creating a much larger peptide pool. Because of the improved sensitivity achieved by both methods, there is less concern about working with small sample sizes or detecting low-frequency T cells. In principle, the two methods could also be applied to study the interactions of tumor antigens with $CD4^+$ T cells, which engage in lower affinity interactions than $CD8^+$ T cells, although this would depend on the ability to rapidly produce class II pMHC monomers, which is more complicated than the process for class I MHC monomers.

The two studies[3,4] provide a template for large-scale, rapid analysis of pMHC antigens that can be widely applied to identify neoantigens and their associated TCRs, to analyze newly identified TCRs for cross-reactivity, and to carry out TCR fingerprinting. This is especially important in the age of TCR-based immunotherapies. Therapies based on T cells transduced with TCRs targeted to a tumor antigen carry a risk of fatal cross-reactivity. The method of Zhang *et al.*[3] can detect and isolate neoantigen-specific T cells with no cross-reactivity to wild-type antigens, while the method of Bentzen *et al.*[4] can screen the identified TCRs against the human proteome for potential cross-reactive antigens. Thus, the two approaches can be used in a complementary fashion to mitigate the risk of TCR cross-reactivity, and they hold exciting promise for preclinical and clinical use.

COMPETING INTERESTS
The authors declare no competing interests.

1. Sewell, A.K. *Nat. Rev. Immunol.* **12**, 669–677 (2012).
2. Birnbaum, M.E. *et al. Cell* **157**, 1073–1087 (2014).
3. Zhang, S. *et al. Nat. Biotechnol.* **36**, 1156–1159 (2018).
4. Bentzen, A.K. *et al. Nat. Biotechnol.* **36**, 1191–1196 (2018).
5. Altman, J.D. *et al. Science* **274**, 94–96 (1996).
6. Newell, E.W. *et al. Nat. Biotechnol.* **31**, 623–629 (2013).
7. Huang, J. *et al. Proc. Natl. Acad. Sci. USA* **113**, E1890–E1897 (2016).
8. Dolton, G. *et al. Front. Immunol.* **9**, 1378 (2018).
9. Bentzen, A.K. *et al. Nat. Biotechnol.* **34**, 1037–1045 (2016).
10. Rodenko, B. *et al. Nat. Protoc.* **1**, 1120–1132 (2006).

Research Highlights

Papers from the literature selected by the Nature Biotechnology editors. (Follow us on Twitter, @NatureBiotech #nbtHighlight)

De novo design of self-assembling helical protein filaments
Shen, H. *et al. Science* **362**, 705–709 (2018)

High prevalence of _Streptococcus pyogenes_ Cas9-reactive T cells within the adult human population
Wagner, D.L. *et al. Nat. Med.* https://doi.org/10.1038/s41591-018-0204-6 (2018)

The opium poppy genome and morphinan production
Guo, L. *et al. Science* **362**, 343–347 (2018)

Pathogen elimination by probiotic _Bacillus_ via signalling interference
Piewngam, P. *et al. Nature* **562**, 532–537 (2018)

Overexpression of Rubisco subunits with RAF1 increases Rubisco content in maize
Salesse-Smith, C.E. *et al. Nat. Plants* **4**, 802–810 (2018)