IMMUNOLOGY

Mind the immuno-connection gap

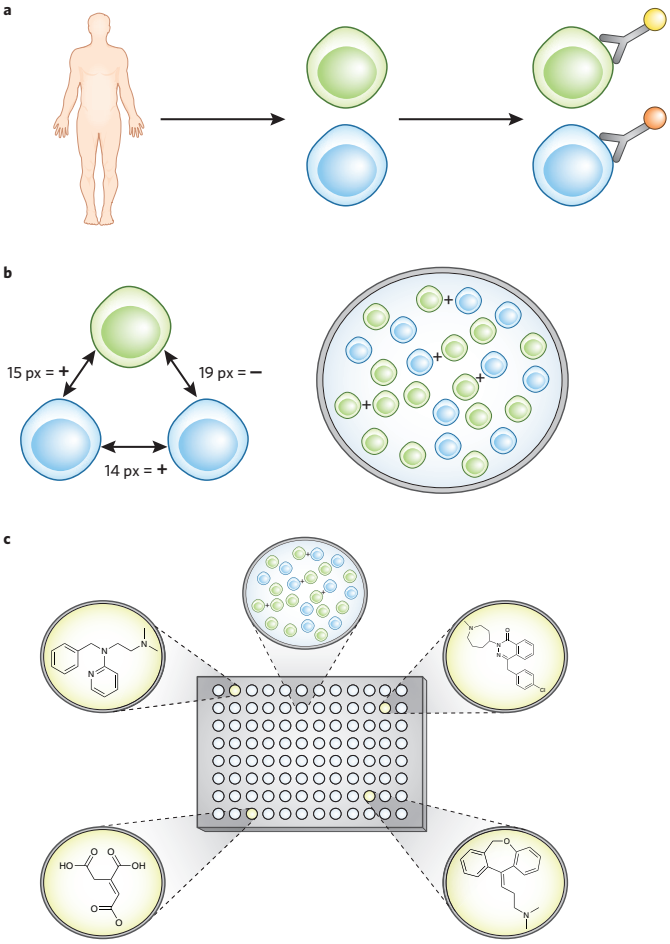
Biologic drugs that modulate the immune system have revolutionized the therapeutic landscape for several selected cancer types. A new study reports an image-based assay system to monitor cell–cell interactions, identifying small-molecule compounds with immunomodulatory capacity.

Wolfgang Link

The human immune system is not optimized to fight cancer cells, as they are 'self' cells with limited immunogenicity. However, our growing understanding of the control mechanisms of the immune system has allowed the development of therapeutic strategies that actively engage the immune system for cancer therapy. A major breakthrough was the discovery of immune checkpoints that limit T-cell immune responses to protect the body against exaggerated immune responses and autoimmunity[1]. In this issue, Vladimer *et al.*[2] develop a high-content-screening (HCS) assay system to identify immunomodulatory agents that act by perturbing cell–cell contacts between immune cells.

Humanized monoclonal antibodies, including ipilimumab and nivolumab, capable of blocking T-cell responses to tumor antigens have entered the clinic in recent years, with unprecedented response rates in advanced melanoma and several other solid tumors. Immuno-oncology drug development also includes small molecules with immunomodulatory effects for anti-cancer therapy either alone or in combination with other treatment modalities[3]. Compared to biologic drugs such as monoclonal antibodies, small-molecule compounds have several advantages including the ease of production and the potential for oral administration. However, there is a consistent lack of screening technology for testing large libraries of small compounds for their immunomodulatory effects in an unbiased manner. The approach taken by the authors is based on the observation that direct cell–cell contacts between immune cells such as cytotoxic T lymphocytes, natural killer (NK) cells, B cells, and dendritic cells are highly dynamic and determine the outcome of the immune response. The interaction between antigen-presenting cells (APC) and T cells, in particular, is essential for cell-mediated immunity[4]. HCS combines the efficiency of high-throughput techniques with the ability of cellular imaging to collect quantitative data from complex biological systems at a single-cell level[5].

For their study, Vladimer *et al.*[2] adapted this technology to interrogate cell–cell contacts *ex vivo* among peripheral mononuclear leukocytes obtained and purified from human peripheral blood. Cells grown in multi-well plates in nonconfluent monolayers were fixed and stained using a nuclear dye and then fluorescently tagged antibodies against extracellular markers specific for different cellular subpopulations for subsequent image acquisition (**Fig. 1a**). A maximal distance of 15 pixels between



Marina Corral Spence/Springer Nature

Figure 1 | High-content screening of cell–cell contacts using automated fluorescence microscopy. (**a**) Mononuclear cells are isolated from peripheral blood and seeded into a clear-bottom 384-well plate to form a monolayer that never fully covers the entire well. Cells are fixed and stained with the nuclear dye DAPI and with fluorescently labeled antibodies to identify cell populations of interest. Automated image acquisition is performed sequentially for each dye channel using HCS equipment. (**b**) Single-cell image analysis is based on DAPI-stained cell nuclei to define nuclear centroids and expanded rings to identify specific cell types labeled by fluorescent antibodies. Cell–cell contacts are defined by a distance between nuclear centroids equal or less than 15 pixels (px). (**c**) Small-molecule compounds and controls are transferred to the 384-well plates and incubated at 37 °C with 5% CO_2 for 36 h.

nuclear centroids of cells was defined to identify cell interaction (**Fig. 1b**). The biological relevance of the assay design was validated by exposure of cells to agents known to alter the interactions between cell populations. As expected, incubation with vesicular stomatitis virus (VSV) or the therapeutic antibodies rituximab and blinatumomab stimulated T-cell-to-APC, NK-cell-to-B-cell or T-cell-to-B-cell interactions, respectively. Conversely, blocking the MHC-II receptor, which is important for the presentation of foreign antigens to T-cells, on the APCs using a specific antibody substantially reduced unstimulated as well as VSV-induced T-cell-to-APC contacts.

The authors then screened a collection of 1,402 diverse compounds, including approved drugs, in VSV-stimulated peripheral blood mononuclear cells from a single healthy donor (**Fig. 1c**). Interestingly, about 10% of all analyzed chemical agents altered cell–cell contacts. In line with their known effect on the immune system, steroidal anti-inflammatory drugs increased VSV infection and decreased $CD14^+$ cell interactions. Furthermore, nonsteroidal anti-inflammatory drugs (NSAIDs), which affect the signaling of the sympathetic nervous system, and cholesterol-lowering drugs were found to be capable of altering cell–cell interactions within the samples. Most intriguingly, the kinase inhibitor crizotinib, approved for the treatment of anaplastic lymphoma kinase (ALK)-positive non-small-cell lung cancer[6], specifically enhanced the interaction between T cells and APCs. The authors present evidence for crizotinib inhibition of the macrophage stimulating 1 receptor, which serves to increase STAT phosphorylation and the expression of the MHC-class-specific transcription factor CIITA, which together with CREB and ATF drives the expression of MHC-I and MHC-II genes. These genes are known to be involved in antigen presentation and peptide processing, loading and trafficking, and are therefore required for an efficient immune reaction to tumor antigens. Further investigations are needed to validate the immunomodulatory effect of the identified chemical compounds in *in vivo* animal models and determine their impact on clinical outcomes.

Several aspects of the work[2] clearly have clinical relevance and might impact future anticancer therapy. The screening platform is suitable for testing large numbers of chemical compounds and can be used as a drug repurposing tool to identify new indications of existing drugs. It can be incorporated into the drug development process to establish immunomodulatory effects of drug candidates in preclinical studies and subsequently guide the design of clinical trials. Rational strategies combining immunotherapy modalities with either chemotherapies, targeted therapies or other immunotherapies are anticipated to produce meaningful synergistic effects[7]. Crizotinib might be an excellent example for this strategy, as it has the potential to boost further immune responses unleashed by checkpoint inhibitors. It is conceivable that many drugs in clinical use produce part of their therapeutic action and side effects by modulating the immune system but have not yet been recognized as immunomodulatory drugs. As these drugs act on the immune system, their mode of action is independent of the specific molecular alterations within a tumor, and hence they might demonstrate activity against a broad range of cancer types. Application of this technology might also lead to the identification of useful therapeutic options for autoimmune diseases or for conditions marked by uncontrolled inflammation. Finally, the fact that the assay uses peripheral blood samples from healthy donors or patients provides the opportunity to predict treatment responses individually and thus the potential to personalize therapy.

Wolfgang Link is in the Department of Biomedical Sciences and Medicine (DCBM), the Centre for Biomedical Research (CBMR), and Algarve Biomedical Center (ABC), University of Algarve, Campus de Gambelas, Faro, Portugal.
e-mail: walink@ualg.pt

Published online 24 April 2017
doi:10.1038/nchembio.2373

References

1. Sharma, P. & Allison, J.P. *Cell* **161**, 205–214 (2015).
2. Vladimer, G.I. *et al. Nat. Chem. Biol.* http://dx.doi.org/10.1038/nchembio.2360 (2017).
3. Murphy, A.G. & Zheng, L. *Hum. Vaccin. Immunother.* **11**, 2463–2468 (2015).
4. Batista, F.D. & Dustin, M.L. *Immunol. Rev.* **251**, 7–12 (2013).
5. Zanella, F., Lorens, J.B. & Link, W. *Trends Biotechnol.* **28**, 237–245 (2010).
6. Shaw, A.T., Yasothan, U. & Kirkpatrick, P. *Nat. Rev. Drug Discov.* **10**, 897–898 (2011).
7. Hoos, A. *Nat. Rev. Drug Discov.* **15**, 235–247 (2016).

Competing financial interests

The author declares no competing financial interests.