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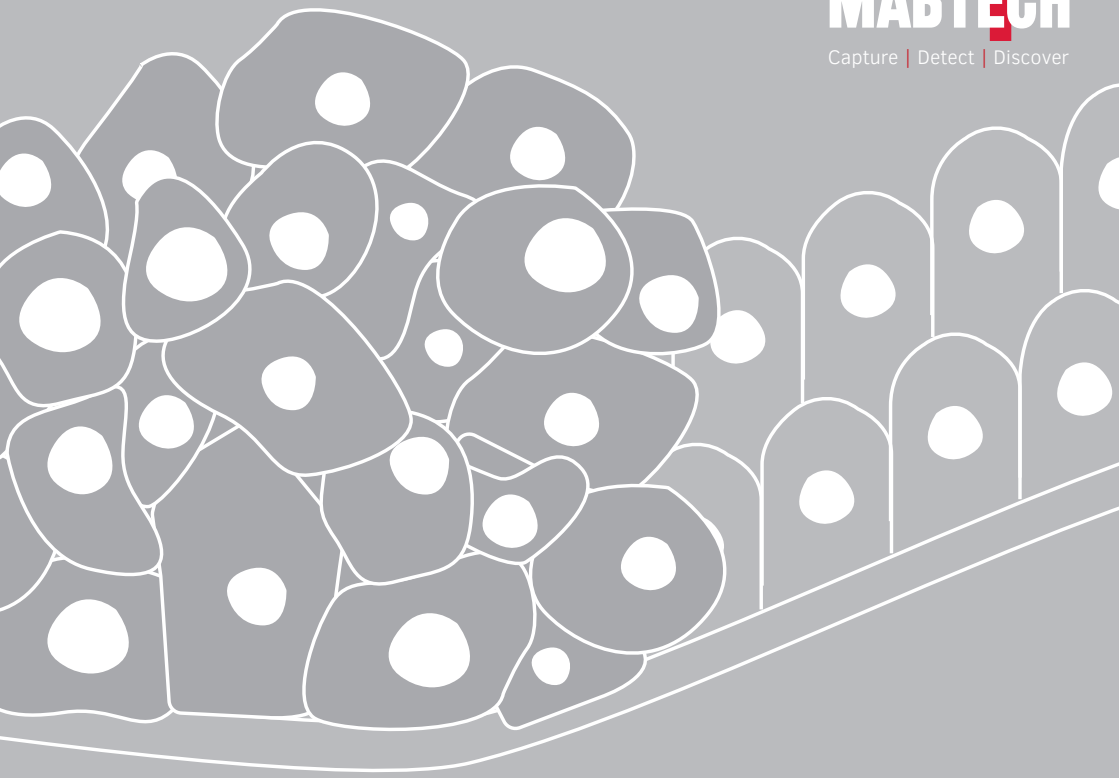


Advance your cancer research by using ELISpot or FluoroSpot

T cells have been identified as promising mediators of cancer immunotherapies. After recognizing their specific antigen, T cells communicate by secreting cytokines. These secreted signalling molecules can be detected using the ELISpot and FluoroSpot techniques.

With detection levels as low as one cell in 250,000, ELISpot and FluoroSpot are ideal for studying **tumor-specific T cells**, typically found at very low levels.

The low levels of tumor-specific immune cells require sensitive detection methods

A major challenge in modern medicine is the development of new and improved cancer therapies. Immunological approaches, termed cancer immunotherapies, have become important alternatives or adjuncts to conventional therapy for several types of tumors *(Upadhaya et al, 2020).*

Most cancer immunotherapies rely on the existence of specific antigens that are selectively expressed or strongly upregulated in tumors compared to healthy tissue. Such antigens are referred to as neoepitopes or tumor-specific antigens and can serve as targets for antibodies as well as cell-mediated cytotoxicity.

Methods of tumor-targeted immunotherapy

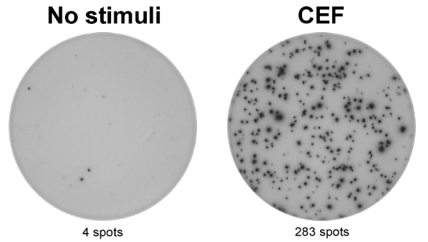
- Cancer vaccine: Therapeutic vaccination with tumor antigens
- TILs: Adoptive transfer of *in vitro*-expanded tumor-infiltrating lymphocytes
- CARs: Adoptive transfer of T cells engineered to produce chimeric antigen receptors
- Check-point blockade: Blocking of immunological tolerance to "release" existing or vaccine-induced anti-tumor responses

Identify T-cell clones reactive to cancer

The ELISpot and FluoroSpot assays can be used to detect and characterize naturally occurring tumor-reactive T cells. These cells are commonly known as **tumor-infiltrating lymphocytes (TILs)** and are suitable mediators of cancer immunotherapy. For this therapeutic approach, a patient's own TILs are expanded *in vitro* and later returned to the patient.

In a case report published in *Nature Medicine, Zacharakis et al (2018)* showed increased efficacy and reduced risk of side effects of a TIL product by identifying and expanding only the neoantigen-reactive clones rather than expanding all extracted TILs. Specifically, the authors first sequenced the patient's breast tumor and identified mutations, i.e. neoepitopes, and subsequently screened the TILs for reactivity to these neoantigens using **IFN-γ ELISpot***. By rapidly expanding only the reactive clones, the authors were able to infuse large numbers of tumor-specific T cells that persisted for several months and eventually eliminated the cancer.

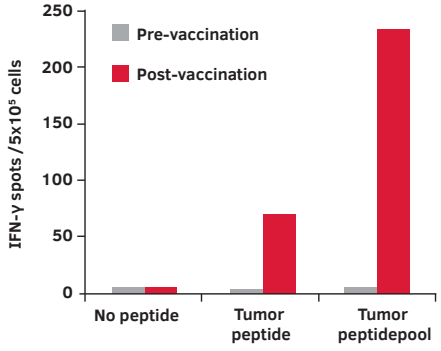
*Human IFN-γ ELISpot^{PRO} (3420-2APT-2)



Negative and positive assay control of a typical human IFN-γ ELISpot on PBMC (No stimuli = cells incubated in medium only; CEF = cells stimulated with pooled peptides from CMV, EBV and Influenza virus).

Monitor immune responses post-cancer vaccination

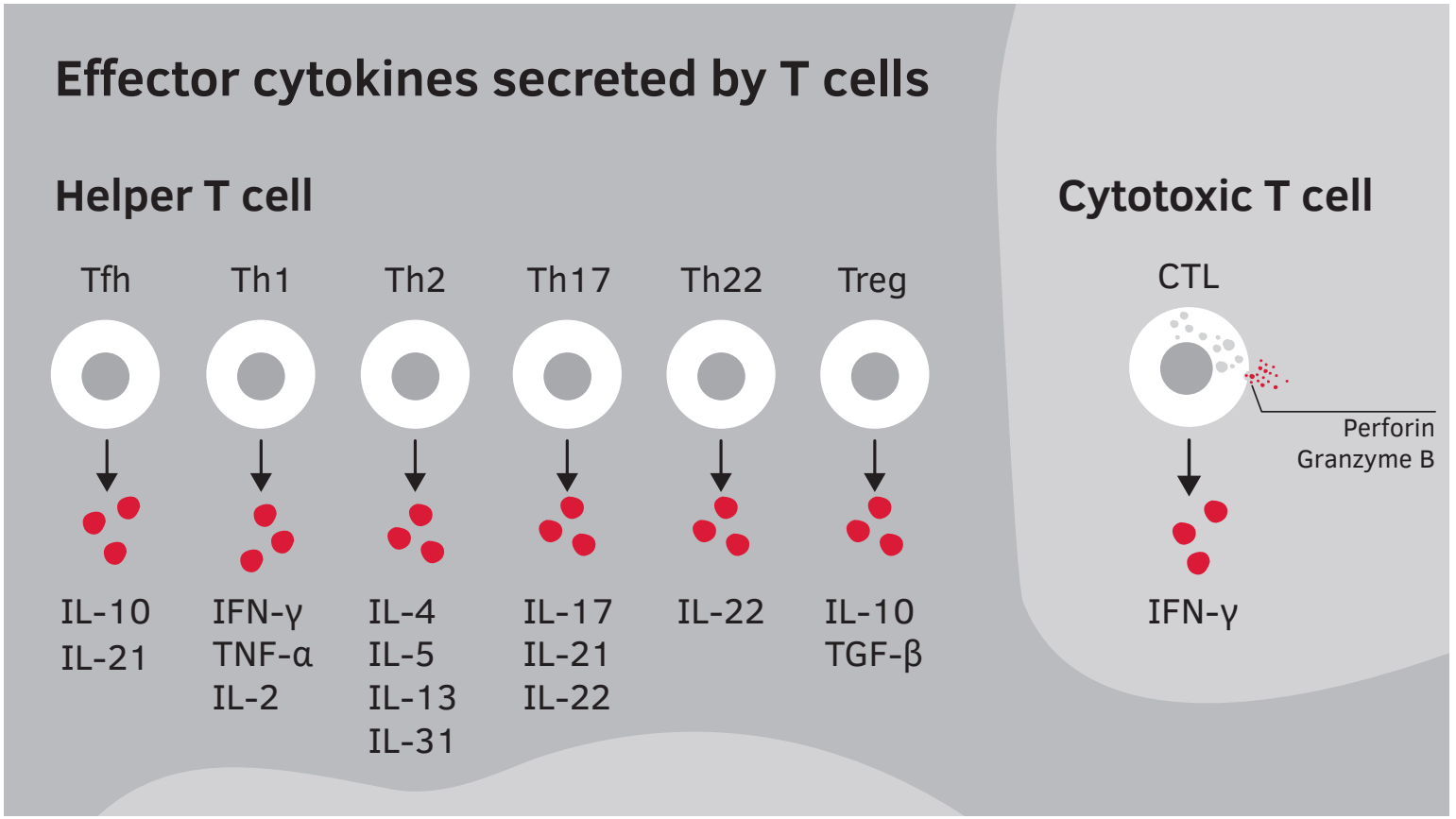
A common issue when detecting TILs is that the cancer epitopes are unknown. Sometimes, the specificity is not important; the T cells that are reactive to the tumor are relevant but the exact antigen is not. An alternative approach is to deliberately skew immune responses toward a **tumor antigen known to be overexpressed** (e.g. MAGE, NY-ESO-1, Gp100, PSA, tyrosinase, and survivin). As with other types of vaccine candidates, whether the cancer vaccine gives rise to an appropriate immune response usually needs to be evaluated. An example of this is shown in the bar-graph below where the induction of antigen-specific T cells was revealed using an **IFN-γ ELISpot*** following vaccination with the tumor-associated antigen survivin *(Lennerz et al, 2014)*.



An IFN-γ ELISpot was used to evaluate a vaccination with a pool of five peptides derived from the tumor-associated antigen survivin. The patient's IFN-γ response to a single tumor peptide and to the tumor peptide pool was monitored before and after vaccination.

Data kindly provided by Dr. V. Lennerz, Johannes Gutenberg University, Mainz, Germany.

*Human IFN-γ ELISpotBASIC (3420-2A)



Find novel tumor antigens by epitope mapping

ELISpot enables the detection of T-cell responses to several antigens in cancer patients prior to vaccination. Importantly, the assay also allows the definition of the most immunogenic epitopes by screening against individual peptides.

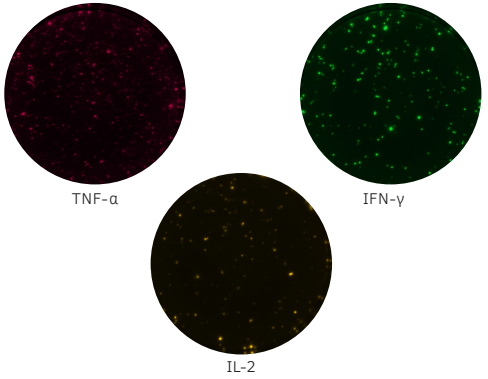
As an example, a group from the Dana-Farber Cancer Institute *(Ott et al, 2017)* used **IFN-γ ELISpot*** assays to demonstrate that personalized neoantigen vaccines generate polyfunctional T-cell responses that persist over time. Specifically, the authors used whole-exome sequencing of DNA from matched tumor and healthy cells to identify somatic mutations, and HLA-binding prediction algorithms to create personalized HLA-binding peptide sequences. Corresponding peptides were synthesized and used as immunogens in patients with previously untreated melanoma. The findings of this study demonstrate that neoepitope vaccines can overcome the challenge of the heterogeneity of tumors and thus minimize tumor escape, and that subsequent checkpoint blockade therapy (the anti-PD1 monoclonal antibody pembrolizumab) may further broaden the repertoire of functional neoepitope-specific T cells.

*Human IFN-γ ELISpot^{PLUS} (3420-4APT-2)

Employ FluoroSpot to detect polyfunctional tumor-reactive T cells

The multiplexing capacity of FluoroSpot enables investigation of the **cytokine-secreting profiles** of tumor-directed T cells. *Fostier et al (2018)* utilized our triple **IFN-γ/IL-2/TNF-α FluoroSpot*** kit to show a more polyfunctional profile, i.e. a higher number of $CD8^+$ T cells secreting all three studied cytokines, in patients with multiple myeloma after treatment with the immunomodulator drug lenalidomide.

However, the expected increased anti-cancer effect of the multipotent $CD8^+$ T cells seemed to be inhibited by a parallel rise in the number of regulatory T cells (Treg). The authors conclude that interventions aimed at reducing Treg expansion during lenalidomide treatment (e.g. by check-point blockade) may further improve the anti-tumor effect.



Example of a human IFN-γ/IL-2/TNF-α FluoroSpot.

*Human IFN-γ/IL-2/TNF-α FluoroSpot^{PLUS} (FSP-010209-2)

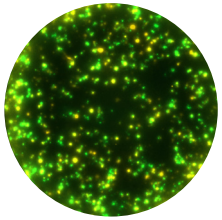
Nobel laureate used our kits

During his research career on check-point blockade, Prof. James P. Allison utilized IFN-γ ELISpot assays to characterize the function of antigen-specific T cells before and after anti-CTLA-4 treatment. For at least three studies, regardless of whether the study was conducted on human subjects or in mouse models, the research group chose ELISpot kits from Mabtech:

- Gregor et al (2004)
- Kitano et al (2013)
- Gubin et al (2014)

Discover T cells capable of killing tumor cells

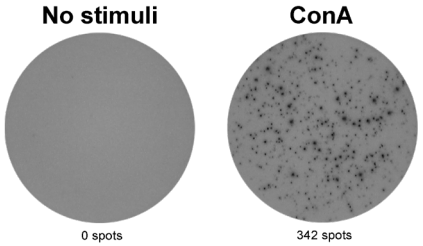
In most immunotherapy studies, IFN-γ has been used as a broad marker for T-cell activation. However, analytes such as Granzyme B and Perforin are expected to better reflect the **cytotoxic capacity** of the T cells. As an example, *Scurr et al (2017)* showed that chemotherapy including cyclophosphamide induced a depletion of Tregs that was mirrored by a boost in cytotoxic effector T cells secreting both **IFN-γ and Granzyme B***.



*Human IFN-γ/GzB FluoroSpot^{PLUS} (FSP-0136-2)

Measure an immune response to less immunogenic tumors

ELISpot is not only applicable for use in clinical trials, but is also widely used in basic research. For example, *Obermajer et al (2018)* presented a **mouse model** in which large numbers of tumor-specific cytotoxic T cells were induced in the spleen and lymph nodes after treatment with an experimental dendritic cell cancer vaccine. By using a **mouse IFN-γ ELISpot*** assay *ex vivo*, the authors detected intratumoral T-cell responses to weakly immunogenic cancers, which might have been difficult using other methods of evaluating tumor-specific immunity.



Negative and positive assay control of a representative mouse IFN-γ ELISpot (No stimuli = cells in medium only; ConA = cells stimulated with Concanavalin A).

*Mouse IFN-γ ELISpot^{PLUS} (3321-4APT-2)

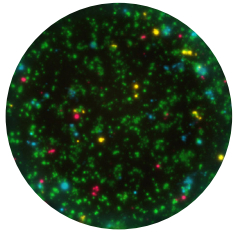
ELISpot and FluoroSpot in cancer research

- Detection and analysis of tumor-specific T cells
- Epitope mapping and identification of tumor antigens
- Monitoring of vaccine-induced T- and B-cell responses

Measure the functionality of other tumor-infiltrating immune cells

Although T cells are believed to be the primary effector cells in the defense against tumors, other cells play pivotal roles and can also be investigated using the ELISpot and FluoroSpot techniques.

For example, *Mullins et al (2019)* studied the functionality of B cells in tumor samples from patients with colorectal cancer. The authors demonstrated that the **tumor-infiltrating B cells** can secrete immunoglobulins by performing an **IgA/IgG/IgM FluoroSpot*** assay with cells isolated from the tumor. Specifically, they found more IgA- than IgG-secreting cells and even fewer IgM-secreting cells. Additionally, they demonstrated a correlation between IgG secretion and number of MHCII-expressing cells (= antigen-presenting cells, including B cells), and noted that Ig secretion was higher in primary tumors compared to metastases. Together, these findings support the hypothesis that tumor-infiltrating B cells have a dual function: Antigen presentation anti-tumor immunoglobulin production.



*Human IgA/IgG/IgM FluoroSpot^{FLEX} (X-06G05R17M-1)

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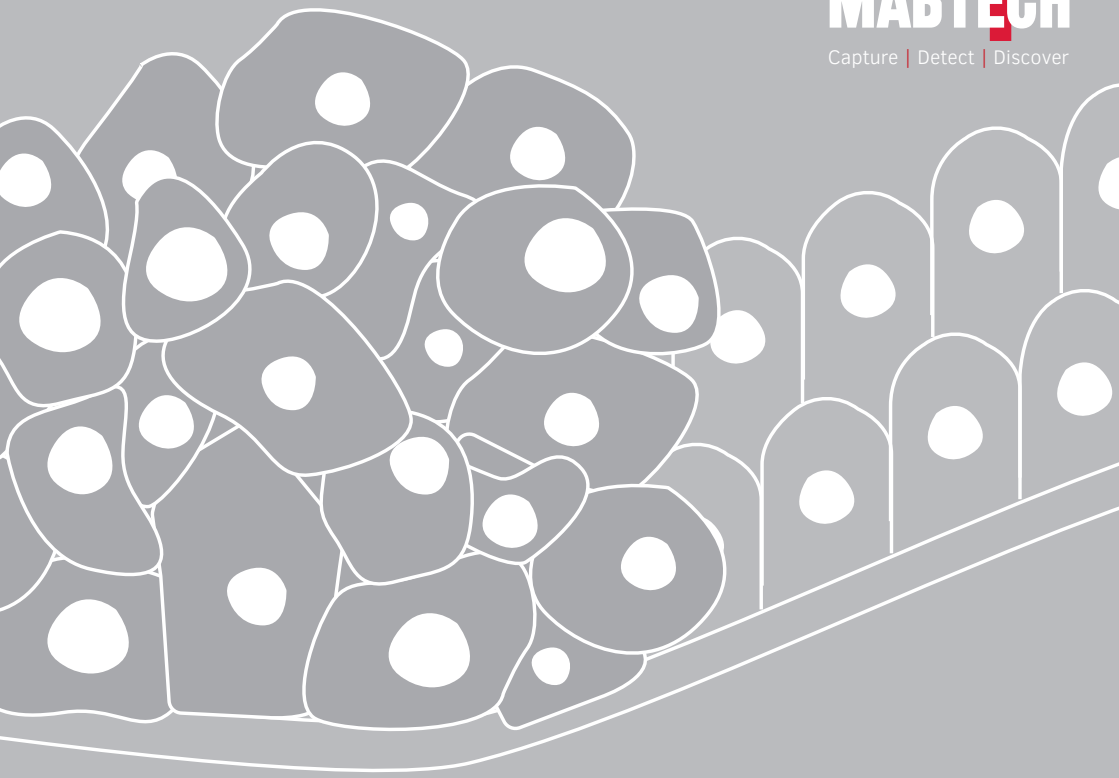

About Mabtech

Mabtech is a Swedish biotech company founded in 1986. Our mission is to aid researchers to reach new frontiers and develop novel therapies, by supplying optimal immunoassays based on high-quality monoclonal antibodies and instruments.

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