Abstract

Immunotherapy with immune checkpoint blockade has been added to the regularly used repertoire of cancer treatments available to many patients supplementing surgery, radio- and chemotherapy. Remarkable efficacy across cancer entities has recently been demonstrated with antibodies directed against Programmed cell death protein 1. However, only a subset of patients respond to immune checkpoint blockade and it has recently been understood that these patients are characterized by a permissive tumor immune microenvironment and pre-existing anti-tumor immunity that is *only* silenced and can easily be reactivated. In contrast, so called *cold tumors* are mostly not susceptible to immune checkpoint inhibition. Thus, rationally developed combination immunotherapies are needed to overcome such primary resistance to immune checkpoint blockade specifically and other immunotherapeutic approaches in general.

Here, I outline the development of a novel, tripartite immunotherapy regimen named TRI-IT. It consists of a combination of systemic immune checkpoint blockade, a local autovaccination with TLR agonists directed at TLR 3, 7 and 9 and a combined adoptive cellular therapy with lymphokine-activated killer cells, cytokine-induced killer cells, Vγ9Vδ2T cells and T cell clones enriched for tumor recognition.

First, I show that a combined adoptive cellular therapy with these four elements in equal parts exhibits synergistic in vitro and in vivo cytotoxicity against multiple cancers compared to single effector cell adoptive cellular therapy. Next, I show that the full TRI-IT protocol eradicates established tumors in two poorly immunogenic mouse models, leads to long term remission in mice and induces humoral and cellular anti-tumor immunity. Lymphodepletion prior to adoptive cellular therapy was necessary for the efficacy of TRI-IT.

Mechanistically, TRI-IT orchestrates a broad anti-tumor immune response with an increased infiltration of effector cells into the tumor, local activation of innate and adaptive immunity and changes in the peripheral cytokine signature. Local injection of one tumor with the three TLR agonists as part of TRI-IT lead to remission of both tumors indicating likely abscopal effects. Finally, the efficacy of TRI-IT was confirmed in lymphoma models, autologous, humanized, and patient-derived lung cancer models and an autochthonous, orthotopic murine lung cancer model.

In conclusion, TRI-IT is a well-characterized and novel combination immunotherapy that could feasibly be evaluated in patients. Importantly, none of the elements of TRI-IT

require prior knowledge of a precise antigenic structure on tumor cells as, for example, bispecific antibodies or chimeric antigen receptor T cells do.