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Targeting two co-operating cytokines efficiently shapes immune responses

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Abbreviations: IFN, interferon; IL, interleukin; scFv, single chain fragment of variable region

For simultaneously mobilizing the adaptive and innate immune system against cancer, we fused interleukin (IL)-2 and IL-12 to generate a dual cytokine moiety that is targeted to neoplastic lesions by an antibody-binding domain. This approach elicits a broader attack of the immune system against cancer than the use of each cytokine alone.

Immunotherapy seems to constitute a valid approach against malignant diseases that are associated with a massive infiltration of innate and adaptive immune cells, leaving malignant cells in a minority. Classical Hodgkin's lymphoma is one example of such a tumor, as $CD30^+$ Hodgkin/Reed-Sternberg tumor cells (H/RS) are surrounded by compact infiltrations of T cells, natural killer (NK) cells and other immune cells, reflecting an ongoing but unproductive immune response.[1,2] Tumor cell-derived factors favor a T_H2 cell polarization and promote the production of circulating interleukin (IL)-10, two factors that are associated with poor prognosis, raising the need to counteract such an immune impairment. The systemic toxicity of most cytokines calls for their local administration. Pro-inflammatory cytokines targeted to tumor cells by means of monoclonal antibodies may constitute a strategy to accumulate the cytokine in a neoplastic lesion, hence achieving therapeutic concentrations and counteracting immune cell mis-polarization. Studies that have previously tested this concept, however, reported an apparent inefficiency of tumor-targeted cytokines to activate adaptive and innate immune responses.

We have recently addressed this same issue by engineering an antibody-cytokine fusion protein that harbors two cytokines, namely IL-2 and IL-12.[3] In this setting, an anti-CD30 single chain antibody (scFv) targeting $CD30^+$ lymphoma cells is linked to the N-terminus of single chain p40-p35 IL-12, which is in turn fused—via the IgG_1 hinge CH2CH3 domain—to the N-terminus of IL-2 (**Fig. 1**). IL-2 and IL-12 are known to co-operate in the activation of T and resting NK cells,[4] as IL-2 functions as a potent inducer of cell proliferation while IL-12 promotes the secretion of multiple cytokines including interferon γ (IFNγ). The synergy between these cytokines results in improved activation of effector cells and lysis of target cells.[4] A recombinant antibody-cytokine fusion protein combining functional IL-2 and IL-12 domains in a single polypeptide chain delivers both cytokines simultaneously to the same target, ensuring the co-operation between these cytokines, which is not always the case when two cytokines are independently targeted to the same site.

Our analyses add several elements to the current understanding of targeted cytokine therapy: (1) The modular composition of the fusion protein employed in our study allows for the integration of additional protein domains. For instance, an IgG_1 Fc domain mediates the dimerization of the molecule by providing cysteines that are available for the formation of disulfide bonds, hence increasing its binding valency. Dimers exhibit a higher avidity for target antigens and a more robust binding to CD30 on the cell surface even in the presence of soluble CD30, whose levels are frequently increased in the sera of $CD30^+$ lymphoma patients. (2) The anti-CD30 scFv antibody specifically targets cytokines to $CD30^+$ cells. The dimeric variant of the fusion protein turned out to be superior than the monomeric form after systemic administration, as it was preferentially retained within the $CD30^+$ tumor tissue. Conversely, the monomeric variant showed a higher capacity to penetrate tissues than the dimeric molecule, resulting in the accumulation within various organs in a merely target-independent fashion. The biological half-life of both formats, however, was very similar. (3) The IL-2-IL-12 fusion protein was superior in activating T cells as compared with targeted IL-2 or IL-12 employed as standalone agents. The synergistic activity of IL-2 and IL-12 became obvious when T cells were withdrawn from stimuli, to keep them in a resting state. The IL-2-IL-12 chimera rescued IFNγ secretion in resting T cells whereas neither IL-2 nor IL-12 was able to do so. NK cells were also activated by the IL-2-IL-12 fusion

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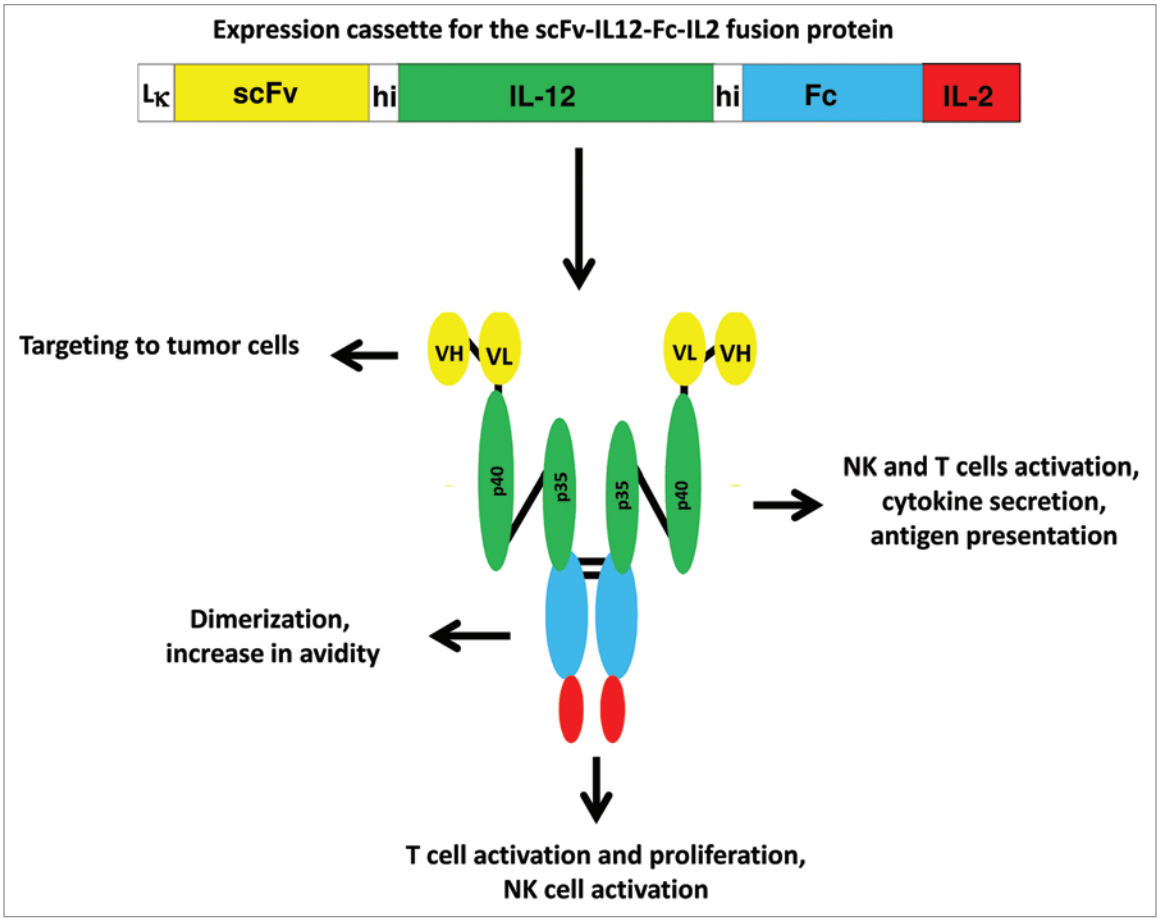

Figure 1. Second generation targeted immunotherapy takes advantage of the combined action of co-operating cytokines. The single chain antibody of the recombinant fusion protein targets the cytokines to CD30$^+$ tumor cells. Interleukin (IL)2 and IL-12 co-operate in the activation of resting T and natural killer (NK) cells; IL-2 provides potent mitogenic stimuli while IL-12 stimulates the secretion of multiple cytokines including interferon γ (IFNγ), resulting in the activation of innate and adaptive immunity against cancer. Fc, human IgG_1 CH2/CH3 domains; hi, human IgG_1 hinge region; Lk, mouse kappa light chain leader peptide; scFv, single chain antibody fragment.

protein, as indicated by an increased cytokine secretion and cytolytic activity. The IL-2-IL-12 chimera remained active upon svFc-mediated binding to the surface of CD30$^+$ target cells. As a consequence, CD30$^+$ cells covered by IL-2-IL-12 were massively attacked by NK cells, resulting in the regression of syngenic tumors transplanted in immunocompetent mice, whereas CD30$^-$ cells—which did not bind the fusion protein—were not attacked.

Another lesson learnt from this study is that our CD30-targeting dual cytokine protein may be suitable to modulate polarized immune responses. CD30 is predominantly expressed on activated T_H2 cells,[5] which are known to accumulate within Hodgkin's lymphoma lesions.[6] Accordingly, targeting T_H2 cells by IL-2 and IL-12 is assumed to shift T cells to a T_H1-polarized response while reverting their hypo-responsiveness in favor of a pro-inflammatory antitumor profile.

Our study demonstrated the potency of co-operating cytokines that are simultaneously delivered to neoplastic lesions. Several tumor-targeted cytokines are currently explored in pre-clinical models as well as in clinical trials, invariably involving the use of a single cytokine. IL-2 targeted to the lymphoma-associated subendothelial matrix of the neo-vasculature resulted in improved lymphoma eradication as compared with un-conjugated IL-2.[7] Used in combination with the CD20-specific antibody rituximab, targeted IL-2 improved the infiltration and activation of immune cells, in particular NK cells, within lymphoma lesions, resulting in the regression of those lesions that did not respond to rituximab treatment alone.

The newly-discovered IL-12 family members IL-23 and IL-27 have been shown to exert anticancer effects in transplanted tumor models.[8,9] As IL-23-induced inflammation can contribute to tumor progression, IL-23 does not appear as a valid alternative to IL-12. Conversely, it may be worth to explore the therapeutic potential of IL-27 in combination with other pro-inflammatory cytokines, like IL-2.

Taken together, our findings demonstrate for the first time that an IL-2-IL-12 dual cytokine specifically targeted to lymphoma lesions improves the local activation of both the adaptive and innate immune system. The simultaneous targeting of both cytokines exerted superior effects as compared with the use of a single tumor-targeted cytokine. We believe that our results support the clinical evaluation of this unique combination of cytokines

for the therapy of malignancies, in particular those that exhibit a pronounced T_H2 polarization, and may stimulate the development of a new generation of tumor-targeted fusion proteins involving synergic cytokines.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.