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Long-lasting antitumor effects provided by radiotherapy combined with the immunocytokine L19-IL2

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Recently, we have shown that radiotherapy (RT) combined with L19-IL2 can induce a long-lasting antitumor effect, dependent on ED-B expression and infiltration of cytotoxic T cells. These findings will be translated to a Phase I clinical study (NCT02086721) in patients with oligometastatic solid tumors. See this link for the animation: http://youtu.be/xHbwQuCTkRc.

Radiotherapy (RT) has several effects in the tumor micro-environment, it causes the release of a broad range of tumor-associated antigens and damage molecular patterns (DAMPs) and upregulates immunomodulatory cell surface and secretory molecules.[1-3] These changes can increase the immunogenicity of the tumor and promote the uptake of tumor antigens by antigen presenting cells that cross-present these tumor antigens to T cells, thereby triggering a cytotoxic T-lymphocyte response resulting in immunogenic tumor cell death.[1,3,4] Unfortunately, this systemic immune response against tumor cells provoked by local tumor RT alone is mostly insufficient to totally eradicate all tumor cells. Therefore, the addition of active immunotherapy (IT) to enhance the effect of RT may be an interesting approach to enhance the therapeutic potential.[5]

The administration of IL2 as IT has the ability to stimulate and expand functional active T cells which has been shown to lead to durable and curative regressions in patients with metastatic melanoma and renal cancer.[6] However, a major drawback of this IT is the occurrence of several toxicities (e.g., capillary leakage syndrome, severe flu-like symptoms and coma) and therefore targeted delivery of IL2 to the tumor could be used to overcome these side effects. The selective delivery of IL2 via the small-immuno-protein L19 to the ED-B domain, a part of the fibronectin of the tumor neovasculature, has shown to have excellent tumor targeting properties and an improved therapeutic index over IL2 treatment alone.[7] Based on the known immunogenic effects of RT on tumors, the immune stimulating effects of IL2 and the possibility of targeted delivery via the immunocytokine L19-IL2, we recently demonstrated that a combination of RT with L19-IL2 causes an enhanced antitumor effect, which is dependent on ED-B expression.[8]

In our preclinical study, we used three immunocompetent mouse tumor models; a colon carcinoma (C51), the Lewis lung carcinoma (LLC) and a breast carcinoma (4T1) model showing respectively high, intermediate and low ED-B expression levels. Combination of RT with L19-IL2 resulted in long-lasting, highly synergistic effects with a cure rate of 75% in the high ED-B expressing model C51. Lowering the RT dose to 5 and 2 Gy decreased the cure rate of the combination therapy to 50% and 8%, respectively. In the LLC model, we observed an additive effect when a single RT dose of 10 Gy was combined with L19-IL2 compared to single treatment arms. In addition, the expression of ED-B seemed to be crucial, since the combination treatment showed no additional therapeutic improvement in the low ED-B expressing model 4T1. Further investigation of tumor immune infiltrate showed a significant increase of $CD8^+$ cytotoxic T cells in C51 and LLC tumors treated with RT and L19-IL2, suggestion a pivotal role for these immune cells. Indeed, when depleting $CD8^+$ cells *in vivo* using the same preclinical experimental setting as in the C51 model, no benefit of the combination therapy could be observed, providing evidence that the complete remission of these C51 tumors is attributed to the presence and activity of cytotoxic T cells.[8] Taken together, we have shown that combination therapy of RT with L19-IL2 can enhance the RT induced antitumor immune reaction, providing a long-lasting antitumor effect when ED-B is present. Since L19-IL2 is proven to be safe in patients, these findings will be translated to a Phase I clinical study (NCT02086721) in patients with oligometastatic solid tumors.

The previous results provide a basis to understand the antitumor immune

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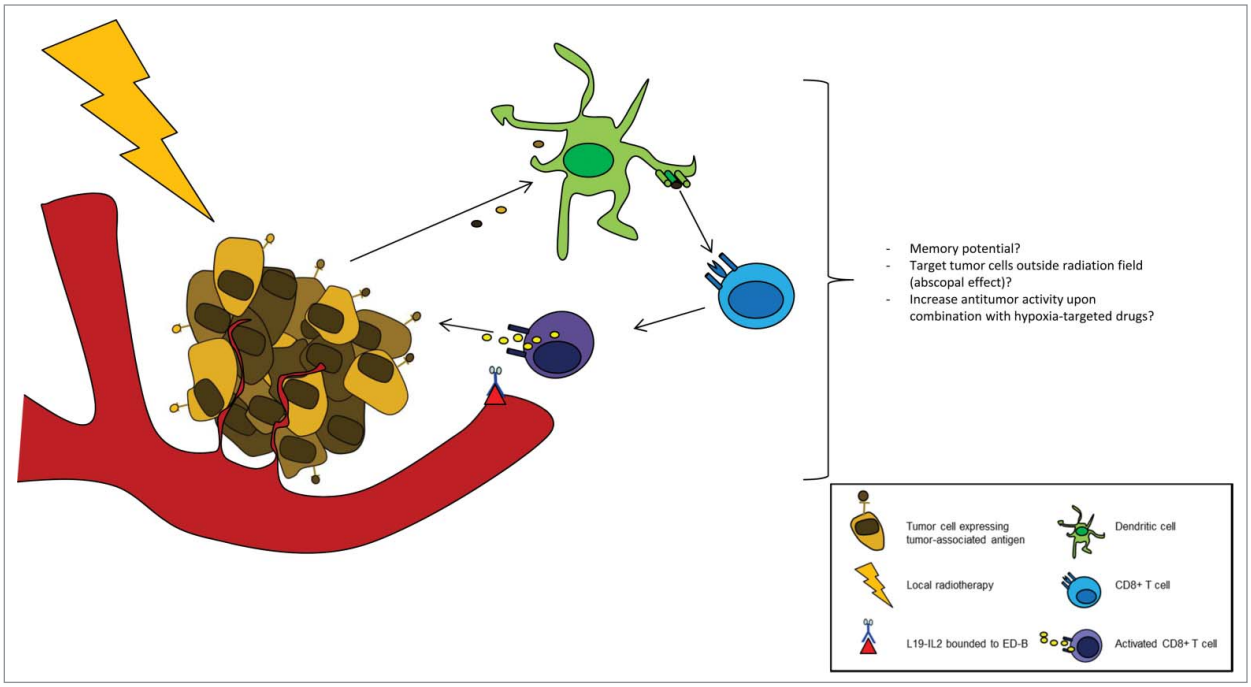

Figure 1. Radiotherapy causes a synergistic and long-lasting antitumor immune response when combined with L19-IL2. This response is dependent on the presence of ED-B in tumor blood vessels and on the presence of cytotoxic T cells inside the tumor.

response after the combination therapy. However, it also provides potential new mechanisms to investigate in pre-clinical setting (**Fig. 1**). One interesting mechanism is the abscopal effect, an antitumor effect observed in tumor cells located outside the radiation field, possibly mediated by the immune system.[9] We hypothesize that the immune system is an important mediator of the abscopal effect, and therefore therapy of RT with L19-IL2 may be an interesting combination to induce and investigate this phenomenon. After RT, a broad variation of tumor-associated antigens is systemically released to function as an "*in situ* vaccination" and therefore, uptake of this antigen mixture by dendritic cells (DCs) might be responsible for the stimulation of a broad cytotoxic T-cell mediated antitumor response, recognizing several antigens expressed on the heterogenic tumor cell population. This can be beneficial in the treatment of multiple tumors and metastases, in and outside the radiation field. Furthermore, an important feature of the adaptive immune system is its memory, which leads to an enhanced response when re-encountering the same antigen. It will be of major interest to investigate if cytotoxic T-cells triggered by RT+L19-IL2 treatment are indeed able to recognize the same tumor-associated antigens and kill the tumor cells after cure. In other words: Do these cytotoxic T-cells have a memory potential? The proliferation of cytotoxic T-cells at the tumor site is of major importance for the development of a strong enough antitumor immune response. However, it is known that hypoxic regions inside solid tumors can prevent an adequate antitumor immune response,[10] possibly explained by the lack of infiltration of cytotoxic T-cells inside the hypoxic areas of treated tumors, suggesting that the combination of this novel treatment (RT+L19-IL2) with anti-hypoxia therapy might be successful.

In conclusion, we have shown a cytotoxic T-cell response after the combination treatment of radiotherapy with L19-IL2 leads to cure in the large majority of treated mice in the C51 model and new research should therefore focus on the cellular and molecular mechanisms underlying this effect as well as on combination treatment which can break the hypoxia immuno-sanctuary.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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