DECREASING TOXICITY OF IMMUNOCYTOKINES BY TRANSIENT AND SELECTIVE INHIBITION OF THEIR INTRACELLULAR SIGNALING ACTIVATION

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Background The use of cytokine-based therapeutics has traditionally been limited by systemic toxicity, but it is now becoming evident that it may be possible to circumvent this problem in a number of ways. Tumor-targeting antibody-cytokine fusions proteins (also called “immunocytokines”) typically allow to administer lower dosages of the payload as a result of the selective localization at the site of disease, thus, improving the therapeutic index. A second aspect relates to the non-linearity of cytokine-mediated toxicity and to the possibility of progressively increasing payload concentration in the tumor. At the clinical level, undesired side effects are often seen when the concentration of cytokines in blood exceeds a certain threshold. In most cases, adverse events disappear when cytokines are cleared from circulation.

Methods Here, we describe an innovative approach to transiently inhibit off-target toxicity of targeted-cytokines, while maintaining their antitumor activity. L19-TNF and L19-IL12 are two clinical-stage tumor-targeted antibody-cytokine fusion proteins that display potent activity by triggering the receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and Janus Kinase 2 (JAK2) intracellular signaling pathways, respectively. Interestingly, small molecule inhibitors and immunocytokines based on antibody fragments have similar half-lives in blood, but only the immunocytokine accumulates at the tumor site for longer periods.

Results In a first study, GSK’963, a potent small molecule inhibitor of RIPK1, was tested in tumor-bearing mice with the aim to reduce acute systemic toxicity associated with TNF signaling. Transient inhibition of RIPK1 allowed the administration of TNF doses, which would otherwise be lethal. The protective effect of GSK’963 did not affect the selective localization of the immunocytokine to tumors. Moreover, L19-murTNF, when given in combination with GSK’963, was still able to induce tumor necrosis and to exert its potent anti-cancer effects. In a second study, tumor-bearing mice receiving L19-murIL12 were pretreated with Ruxolitinib (Jakafi®), a commercially available JAK2 inhibitor. The addition of Ruxolitinib could significantly improve the tolerability profile without affecting the anti-cancer properties of the immunocytokine. The safety profile was monitored following the body-weights of the animals and by measuring the interferon-γ (IFNγ) levels in blood at early time points after the intravenous injection of L19-murIL12.

Conclusions Our results are of clinical relevance, as patients treated with targeted cytokines L19-TNF and L19-IL12 could potentially benefit from judicious combinations treatments using GSK’963 or Ruxolitinib respectively.