Interleukin-12 Message in A Bottle

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SUMMARY

IL12 is a very potent cancer immunotherapy agent, but is difficult to harness safely if given systemically. Local gene transfer aims to confine the effects of IL12 to malignant tissues, thus avoiding toxicity. Lipid-nanoparticle mRNA achieves IL12 expression and efficacy in mouse models, opening the way to an ongoing trial.

See related article by Hewitt et al. p. 6284

In this issue of *Clinical Cancer Research*, Hewitt and colleagues provide compelling results on the preclinical antitumor efficacy of lipid nanoparticles containing mRNA encoding for IL12 (1). IL12 is a dimeric cytokine and a single chain version of the moiety has been constructed with a flexible linker. The immunotherapy agent for intratumoral delivery has been optimized as a result of several lines of research. First, the lipid formulation is optimal for gene transfer of tumor cells and other cells in tumor stroma (ref. 2; AACR 2020 abstract CT032). Second, the RNA construction has been optimized to attain maximal local expression encompassing nontranslated sequences to enhance translation and persistence. Furthermore, such mRNA is devoid of activity on pathogen-associated molecular pattern receptors that via type I IFN responses would otherwise compromise the expression of the transgene. Having said so, a certain level of local IFNα/β might be actually important for an optimal antitumor immune response (3). Third, given the fact that systemic exposure to IL12 could be undesirable above certain levels, the construct incorporates a target miR-122 sequence, so the expression in the liver of leaked agent from the injected tumors will not pose a safety problem. This agent shows impressive efficacy against transplanted mouse tumors, a fact that is not surprising because multiple approaches of IL12 local gene transfer have attained efficacious preclinical activity. Interestingly, prominent therapeutic synergistic effects are found when combining the local IL12 mRNA transfer with systemic PD-L1 blockade. Most promisingly, this treatment leads to measurable efficacy on distant noninjected tumors (**Fig. 1**).

IL12 has a long history as an immunotherapy agent, but its use is constrained by its relatively narrow on-target therapeutic window. IL12 acting on its receptors (IL12Rβ1/IL12Rβ2) triggers high levels of IFNγ, which is its main downstream mediator not only for efficacy, but also for systemic toxicity (4). Recombinant protein trials given intravenously were halted due to serious safety problems. In this scenario, a quest to harness the potent immunobiology of this cytokine for cancer immunotherapy was launched with multiple approaches having the common goal of attaining tumor-localized and transient gene expression, that overall had impressive efficacy in mouse models (4), but insufficiently translated to the clinic in monotherapy approaches in terms of efficacy. At the beginning of this quest, viral vectors dominated the scenario, but it is nonviral gene transfer approaches that are currently the most promising (**Fig. 1**).

A relatively simple strategy has been the intralesional injection of an expression plasmid encoding IL12 (tavokinogene telseplasmid) into cutaneous or subcutaneous melanoma lesions followed by *in vivo* electroporation to greatly augment gene transfer. This strategy has attained single agent activity (ORR = 35.7%) and promising results upon combination with pembrolizumab (ORR = 41%) in a single-arm clinical trial (5). Importantly, tumors from these patients showed the expected IL12-attributable changes in T-cell infiltrates, TCR sequencing and activation of Th1 and CTL antitumor immune responses. Results from an on-going clinical trial are eagerly awaited, analyzing the local electroporation of tavokinogene telseplasmid in combination with systemic pembrolizumab (NCT03132675).

Although there is extensive experience on repeated intralesional delivery into tumors beyond the skin, the need for the electroporation procedure might pose obstacles for visceral metastases. Using optimized lipid-nanoparticle mRNA therefore offers advantages including the possibility of combining several immunostimulatory genes. In this regard, a similar approach collectively injecting mRNAs for OX40L, IL23, and IL36γ has also shown consistently effective results in preclinical mouse models augmenting antitumor immunity (2).

In the same regard, lipid formulation virtuosity seems to be conducive to the impressive levels of local mRNA expression (2) already observed in clinical trials (AACR 2020 abstract CT032). Indeed, ionizable cationic lipids are key for optimal cytosolic delivery of therapeutic mRNAs following endosomal disruption of the lipid nanoparticles.

Viral vectors might still have a future in IL12-based immunotherapy because of enhanced delivery, although repeated administration might be compromised by viral immunogenicity and other factors. In fact, multiple viral vectors encoding IL12 are under advanced preclinical development and going forward to clinical programs with an intense safety focus. Alternation of viral and nonviral IL12 local gene transfer approaches is not inconceivable and, although adding a layer of complexity, it might offer optimal therapeutic results.

In a hybrid viral/nonviral approach RNAs encoding for self-replicating IL12 encoding RNA alpha-virus amplicons based on Venezuelan equine encephalitis virus have recently shown impressive results in mouse models. Local intratumoral delivery is also achieved in this case by conjugation in lipoplexes (3).

Important unresolved questions for these local IL12 approaches are how often intratumoral administration is needed and whether

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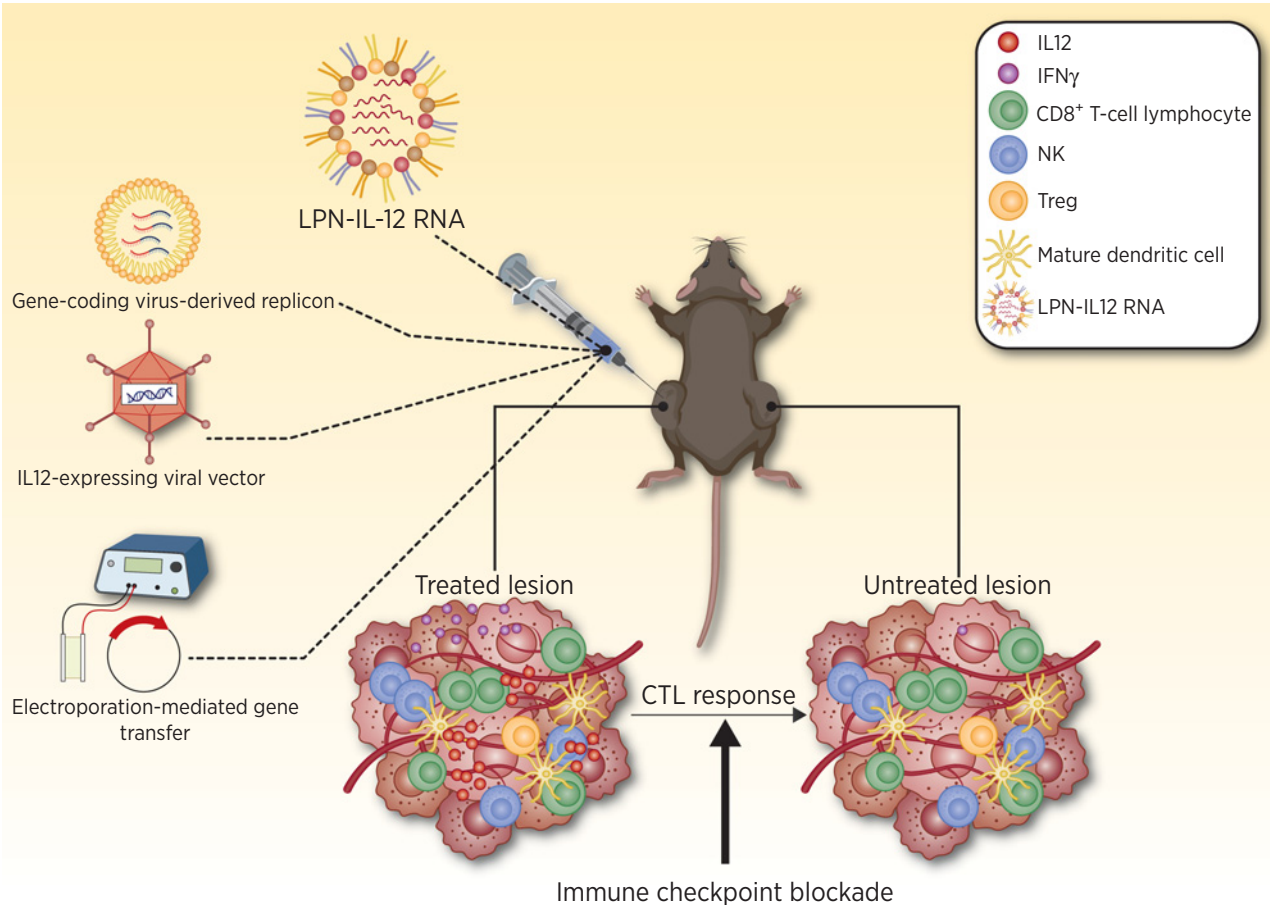

Figure 1.
Schematic representation of local IL12 gene transfer approaches and its envisioned modes of action. IL12 local gene transfer with viral and nonviral vectors seeks to locally foster antitumor immunity with the intention to unleash mechanisms that would impact noninjected tumor lesions. Multiple cellular and molecular immune system elements will be at play and the strategy benefits from synergistic combinations, such as those with checkpoint inhibitors. Adapted from an image created with BioRender.com.

intermittent exposure is advisable to avoid desensitization of the IL12 receptor. The immunobiology of the IL12–IFNγ axis is fascinating but, in its intricacy, it turns on compensatory mechanisms such as PD-L1, IDO-1, or SOCS-1 that require suitable combination partners to tackle the negative influence of the compensatory mechanisms on the overall antitumor immune response. It is very important to address whether IL12 is only needed in one of the tumor lesions or some systemic exposure is also needed, as experiments with tumor-tethered forms of IL12 seem to suggest (1, 3). Accordingly, delivery, schedule, combinations and biodistribution will be the key words for future progress (1). The idea that a tonic signal of IL12 even at low levels is needed to keep the immune system able to mount Th1 and CTL responses is probably correct and offers opportunities for intervention and testable hypotheses because IL12 activity can be quantitatively assessed by STAT-4 phosphorylation levels.

In the clinical trial arena, IL12-based agents are slowly making their way forward. A systemically given IL12 immunocytokine (NHS-IL12) that targets extracellular DNA to achieve selective biodistribution to the tumor seems to provide a reasonable therapeutic window and is being tested in combination with avelumab (NCT02994953).

However, the beauty of local release as the means of turning tumors into their own vaccines is the most elegant approach and mRNA might prove to be especially well suited for this purpose. A clinical trial based on the approach preclinically reported in this issue of *Clinical Cancer Research* is already ongoing. There are good reasons for optimism in particular regarding its combination with the anti-PD-L1 mAb durvalumab (NCT03946800).

Disclosure of Potential Conflicts of Interest

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