Title

Interleukin-12 message in a bottle

Authors

Assunta Cirella[1,2], Pedro Berraondo[1,2,3], Claudia Augusta Di Trani[1,2], Ignacio Melero[1,2,3,4,*]

Affiliations

[1]Program of Immunology and Immunotherapy, Cima Universidad de Navarra, Pamplona, Spain.

[2]Navarra Institute for Health Research (IDISNA), Pamplona, Spain.

[3]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Spain.

[4]Department of Immunology and Immunotherapy, Clínica Universidad de Navarra, Pamplona, Spain

*Contact information for corresponding author:

Ignacio Melero, M.D. Ph.D.

Cima Universidad de Navarra

Avenida Pio XII 55

31008 Pamplona Spain

Tfln: +34948194700 e-mail: imelero@unav.es

Running title

mRNA IL-12 in cancer immunotherapy

Conflict of interests

IM reports advisory roles with Roche-Genentech, Bristol-Myers Squibb, CYTOMX, Incyte, MedImmune, Tusk, F-Star, Genmab, Molecular Partners, Alligator, Bioncotech, MSD, Merck Serono, Boehringer Ingelheim, Astra Zeneca, Numab, Catalym, Bayer, Servier, and PharmaMar, and research funding from Roche, BMS, Alligator, and Bioncotech. PB reports advisory roles with Tusk and Moderna, research funding from Sanofi, and Bavarian Nordic and speaker honoraria from BMS, MSD, Novartis, Boehringer Ingelheim, and AstraZeneca. The rest of the authors have no conflict of interest to declare.

Summary

IL-12 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 IL-12 to malignant tissues, thus avoiding toxicity. Lipid-nanoparticled messenger RNA achieves IL-12 expression and efficacy in mouse models, opening the way to an ongoing trial.

Main

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 IL-12 (1). IL-12 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 ((2) and AACR 2020 abstract CT032). Second, the RNA construction has been optimized to attain maximal local expression encompassing non-translated 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 interferon-α/β might be actually important for an optimal antitumor immune response (3). Third, given the fact that systemic exposure to IL-12 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 which is not surprising since multiple approaches of IL-12 local gene transfer have attained efficacious preclinical activity. Interestingly, prominent therapeutic synergistic effects are found when combining the local IL-12 mRNA transfer with systemic PD-L1 blockade. Most promisingly, this treatment leads to measurable efficacy on distant non-injected tumors (**Fig. 1**).

IL-12 has a long history as an immunotherapy agent but its use is constrained by its relatively narrow on-target therapeutic window. IL-12 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 non-viral gene transfer approaches which are currently the most promising (**Fig. 1**).

A relatively simple strategy has been the intralesional injection of an expression plasmid encoding IL-12 (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 IL-12-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-nanoparticled mRNA therefore offers advantages including the possibility of combining several immunostimulatory genes. In this regard, a similar approach collectively injecting mRNAs for OX40L, IL-23 and IL-36γ 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 IL-12-based immunotherapy because of enhanced delivery, although repeated administration might be compromised by viral immunogenicity and other factors. In fact, multiple viral vectors encoding IL-12 are under advanced preclinical development and going forward to clinical programs with an intense safety focus. Alternation of viral and non-viral IL-12 local gene transfer approaches is not inconceivable and, although adding a layer of complexity, it might offer optimal therapeutic results.

In a hybrid viral/non-viral approach RNAs encoding for self-replicating IL-12 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 IL-12 approaches are how often intratumoral administration is needed and whether intermittent exposure is advisable to avoid desensitization of the IL-12 receptor. The immunobiology of the IL-12-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 IL-12 is only needed in one of the tumor lesions or some systemic exposure is also needed, as experiments with tumor-tethered forms of IL-12 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 IL-12 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 since IL-12 activity can be quantitatively assessed by STAT-4 phosphorylation levels.

In the clinical trial arena, IL-12-based agents are slowly making their way forward. A systemically given IL-12 immunocytokine (NHS-IL-12) 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 monoclonal antibody durvalumab (NCT03946800).

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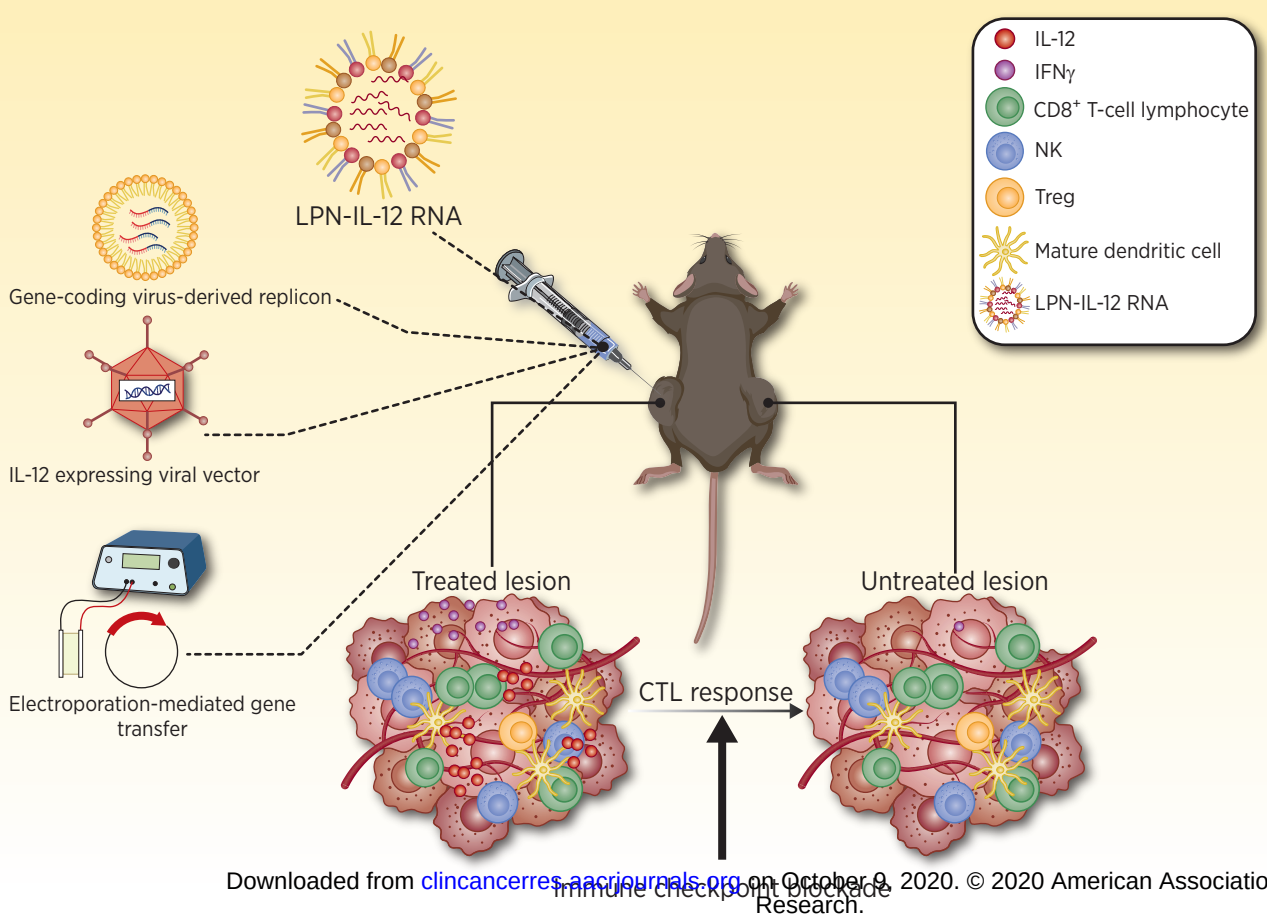
Figure legend

Figure 1. Schematic representation of local IL-12 gene transfer approaches and its envisioned modes of action. IL-12 local gene transfer with viral and non-viral vectors seeks to locally foster antitumor immunity with the intention to unleash mechanisms that would impact non-injected 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.



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