Immune-checkpoint blockade aptamers as a feasible clinical alternative to monoclonal antibodies

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The recent U.S. FDA approval of monoclonal antibodies against CTLA4 (ipilimumab) and PD1 (nivolumab and pembrolizumab), together with the increasing number of clinical trials for new immune-checkpoint blockade antibodies in monotherapy and in combinations, have emphasized the therapeutic potential of using immunomodulatory antibodies to elicit an effective protective immunity for cancer immunotherapy. However, the treatment of cancer patients with immune-checkpoint blockade antibodies, not devoid of toxicity, is associated with severe auto-inflammatory immune responses. It is urgent to identify new therapeutic immune-checkpoint blockade reagents with more manageable side effects. The chemically synthesized single-strand oligonucleotide aptamers have substantial advantages versus antibodies in terms of cost production and handling side effects.

Keywords: Aptamer; Cancer Immunotherapy; Immune-checkpoint blockade; PD1; CTLA4; TIM3

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Aptamers are single-strand oligonucleotides of RNA or DNA acquiring complex tertiary structures that allow them to interact with their cognate receptor with high affinities and specificities. They have been described for the first time by Tuerk et al. and Elligton et al. in 1990 [1,2]. Over the last few years, aptamers have been gaining a niche in therapeutics [3]. Indeed, aptamers have started to be applied in cancer immunotherapy lately with the first 2′F′-RNA aptamer against CTLA4 described by Gilboa’s group [4]. Since then, other aptamers for immune-checkpoint receptors have been discovered; recently, an anti PD1 DNA aptamer [5] and a 2′F-RNA aptamer anti TIM3 by my team [6]. All these immune-checkpoint blockade aptamers have shown potent therapeutic effect in different types of tumors.

The era of cancer immunotherapy has come to prominence with the unprecedented results in clinical trials with immune-checkpoint blockade antibodies [7, 8]. These strategies are aimed to facilitate the induction of new antitumor immune responses or to unleash the dormant ones. Among the immune-checkpoint blockade strategies, we could divide those receptors that have a certain role in facilitating the priming of the immune response such as CTLA-4 that regulates the amplitude of T-cell activation in the early stages, and those that are aimed at reverting T-cell exhaustion (PD1, TIM3 and LAG3 among others). The
blockade of these types of receptors (CTLA4 and PD1) has been tested in clinical trial separately and in combination, and although both have a clinical benefit, T-cell exhaustion reversion by PD1 blockade has a superior clinical outcome [8]. The results from clinical trials, contrary to what is observed in tumor murine models where CTLA4 blockade is more potent than PD1 [8, 9], indicate than in humans T-cell exhaustion is one of the major causes for lack of efficient tumor immunity.

CTLA4 acts as a rheostat damping the T-cell activation, CTLA4 blockade reduces the threshold of T-cell activation possibly facilitating the priming of tumor reactive T cells with lower TCR affinity [10]. The role of CTLA4 on Treg has been more argued [10]. The first selected immune-modulatory aptamer was against the receptor CTLA4; the aptamer binds with high affinity (Kd 30 nM) to CTLA4 and not to CD28. The 2'-F'RNA CTLA4 aptamer was truncated from 80 nucleotides to 35 nucleotides, maintaining the binding affinity and the blocking capacity. The aptamer was multimerized into a tetramer to be used in tumor protective murine studies with a comparable efficacy than the monoclonal antibody [4].

PD1-PDL1 axis has been underscored as the major receptor responsible for eliciting T-cell exhaustion. A large amount of tumor infiltrated lymphocytes (TILs) express PD1 indicating a high stage of T-cell exhaustion in the tumor. The outcome of clinical trials with anti-PD1 blockade antibodies nivolumab and pembrolizumab as monotherapy shows a potent clinical response with fewer side effects in comparison with CTLA4 blockade antibodies (ipilimumab) [8, 9]. Prodeus et al. have recently selected a DNA-blocking aptamer against PD1 receptor by SELEX with a Kd of 167 nM; the aptamer was PEGylated to increase the size and half-life in vivo. They have validated the antitumor effect of the PEGylated PD1 blocking aptamer in vivo in colon carcinoma tumor model [13].

TIM3 and LAG3 are other receptors that also play a very important role in T-cell exhaustion. Immune-checkpoint reagents against TIM3 and LAG3 are second to PD1 and PDL1 blockade antibodies [11]. In pre-clinical studies, the blockade of the PD1: PDL1 axis in combination with any of the other two receptors (TIM3 or LAG-3) has been shown to exhibit synergetic antitumor effects [12]. The identification of tumor-reactive TILs has been identified as the lymphocytes that co-express the three receptors (PD1, TIM3 and LAG3) [13]. The concurrent expression of TIM3, LAG3 and PD1 is characteristic of the most exhausted T cells [14]. Our team has recently selected a blocking aptamer against TIM3; the aptamer displayed an affinity of 22 nM to the cognate receptor. TIM3 blocking aptamer in combination with anti-PDL1 antibody elicit a potent antitumor response in colon carcinoma CT26 orthotropic tumor model [6].

In spite of the advances of immune-modulatory aptamers monoclonal antibodies are still the most extended reagents used in cancer immunotherapy. The use of antibodies for therapeutic intervention, although showing great results in clinical trials, is still far from optimal. Monoclonal antibodies are cell-based products, implying a higher manufacture cost and a complex regulatory approval to be used in the clinic; that translates into extremely high market prices. Considering that immunotherapy is leading towards multipronged approaches aimed at combining blockade or stimulation on several immune receptors, the prices for this type of treatment would escalate with each new monoclonal antibody added to the combo. Also, antibodies as protein products are not devoid of antigenicity; recurrent administration of protein derived therapeutic agents, can elicit a T-cell dependent neutralizing antibodies, reducing the therapeutic benefit, especially in case of tumor recurrence and re-initiation of the protein based treatment. And finally the use of immune-modulatory agents on inhibitor or stimulatory receptors can trigger severe side effects usually associated with exacerbated auto-inflammatory immune responses [15]. Monoclonal antibodies, due to theirFc region, display a long half-life in the blood stream, ranging from weeks to months. Therefore, in case of side effects, antibodies will persist in the blood stream, exacerbating the auto-inflammatory damage while the only clinical maneuver is to treat the patients with potent immunosuppressive drugs until the side effects diminish. The treatment with immune-suppressants to counteract the side effects could also hamper the benefit of the cancer immunotherapy approach. So it is important to use a therapeutic agent that could be rapidly neutralized in case of side effects. The identification of alternative therapeutics agents that could overcome all these caveats would be of great interest in the

**Table 1. Main differences of Aptamers over Monoclonal Antibodies for Cancer Immunotherapy**

<table>
<thead>
<tr>
<th>Advantage of aptamer versus antibodies</th>
<th>Aptamers</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simpler and lower cost of production</td>
<td>Chemically synthesized reagents</td>
<td>Cell-derived products</td>
</tr>
<tr>
<td>Easier toxicity manageable</td>
<td>Short half-life</td>
<td>Long half-life</td>
</tr>
<tr>
<td>Reduce antigenicity</td>
<td>Universal antidote available</td>
<td>No antidote available</td>
</tr>
<tr>
<td>Higher penetration rate in the tumor</td>
<td>Non-protein based products, it is not expect to elicit T-cell dependent humoral immune responses</td>
<td>Proteins can elicit neutralizing humoral immune response against the idiotype</td>
</tr>
<tr>
<td></td>
<td>Small molecules (8-15 KD size)</td>
<td>Large molecules (150 KD size)</td>
</tr>
</tbody>
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field of cancer immunotherapy.

Based on the results from the last years, aptamers are presented as clinically feasible reagents in cancer immunotherapy with some differences from monoclonal antibodies. Both aptamers and monoclonal antibodies exhibit similar affinities and specificities. Aptamers are chemically synthesized reagents and not cell-derived products, which is an important advantage for good manufactured production, with a direct impact on production cost, offering more competitive prices for cancer patients. Aptamers are also nearly devoid of antigenicity, they are not proteins and, therefore, the chances to elicit neutralizing humoral responses are considerably reduced. Aptamers are also smaller molecules with 8-30 KD of molecular size versus 150 KD for monoclonal antibodies; this smaller size implies a higher penetration rate within the tumor, improving the accessibility to intra-tumor infiltrated lymphocytes. The smaller size of the aptamer also affects its half-life in blood, ranging from few hours to one day depending on the aptamer versus several weeks in the case of antibodies. These shorter half-lives could be a caveat as a higher number of injections might be necessary, but managing toxic side effects associated to the treatment would be an advantage. As soon as the side effects revealed and the treatment is stopped, the aptamer will be cleared out in few hours. Besides, aptamer function could be blocked almost instantly within few minutes upon the injection of a universal antidote [16].

Conflicting interests

The authors have declared that no conflict of interests exist.

Abbreviations


References