Clinical Cancer Research

Lost in Translation: Deciphering the Mechanism of Action of Anti-human CTLA-4

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Despite a number of preclinical studies demonstrating that the activity of anti-CTLA-4 antibodies in murine models of cancer relies on effector T-cell activation and regulatory T cell depletion, the activity of the clinical antibodies remains controversial. To decipher such mechanisms is critical to the development of novel and more potent immunotherapies.

See related article by Sharma et al., p. 1233

In this issue of *Clinical Cancer Research*, Sharma and colleagues (1) use samples from patients with melanoma, prostate, and bladder cancer in an attempt to determine whether anti-human CTLA-4 antibodies promote depletion of tumor-infiltrating regulatory T cells (Treg) in the clinical setting. When comparing stage-matched untreated to anti-CTL antigen 4 (anti-CTLA-4; ipilimumab)-treated samples, the authors observed a higher density of effector CD4 and CD8 effector T cells (Teff) in ipilimumab-treated samples. Contrary to prior findings from a number of preclinical mouse models, the authors observed an increased rather than reduced density of $Foxp3^+$ Tregs. Similar findings were obtained when analyzing paired tumor samples in a cohort of patients with melanoma treated with a different anti-CTLA-4 antibody (tremelimumab), reigniting the controversy around the *in vivo* mechanism of action of anti-CTLA-4 antibodies in the clinic.

CTLA-4 was the first immune inhibitory checkpoint identified by Allison and colleagues, who proposed it as a potential target for agents aiming to augment the anticancer activity of the immune system. More than 20 years after the seminal proof-of-principle experiments in mice (2), a large phase III clinical trial formally demonstrated the efficacy of anti-CTLA-4 antibodies (ipilimumab) against late-stage metastatic melanoma (3), reinvigorating the field of immunotherapy and opening the minds and doors of the cancer research community to both anti-CTLA-4 and the avalanche of novel immunotherapy agents that followed.

Defining the mechanism of action of anti-CTLA-4 antibodies has, however, proven difficult at both the preclinical and clinical level. While the initial work in preclinical models demonstrated a clear impact on the Teff compartment, further analysis identified Tregs as an additional key target (4). In mice, the checkpoint-blocking activity of anti-CTLA-4 drives the expansion of both Teff and Treg compartments, while the ability of the antibody to engage with activating Fcγ receptors (FcγR) on innate cells drives depletion of CTLA-4^{hi} Tregs. This dual activity promotes a change in the intratumor immune balance, favoring the accumulation of Teffs and driving tumor rejection (5–7). Several groups have now confirmed the impact of anti-CTLA-4 on both Teff and Treg compartment in mouse models, supporting a series of new studies evaluating whether the same mechanisms are active in the context of the clinically available antibodies. Whether these antibodies [ipilimumab (human IgG1 with predicted depleting activity) and tremelimumab (human IgG2 with low-predicted depleting activity)], are able to promote Treg depletion *in vivo* is not only relevant to our basic understanding of cancer immunology and immunotherapy, but also critical to the potential development of a next generation of anti-CTLA-4 antibodies with enhanced Treg-depleting and antitumor activity.

While simple in principle, the translation of murine mechanistic findings to the clinical setting is complicated by a number of elements significantly differing between the two "models." Genetic homogeneity combined with the ability to synchronize tumor challenge and to serially monitor immune infiltrates within the whole tumor greatly facilitates mechanistic studies in mice, but cannot be easily recapitulated in the context of clinical trials. Experiments assessing Treg depletion in mice are performed in well-characterized models where the kinetics of tumor growth and response to anti-CTLA-4 are clearly defined and, importantly, where the whole tumor is collected for analysis of immune infiltrates. Genetic homogeneity provides a mirrored version of "serial" sampling, enabled by evaluation of parallel cohorts of mice assessed at different time points. Clinical studies are limited to a single, or in the best cases to a couple biopsies, which due to intratumor heterogeneity provide a partial and heterogeneous representation of the whole tumor lesion.

The work of Sharma and colleagues helps to highlight some of the further difficulties in the field. The snapshots that can be evaluated from clinical studies, particularly if not performed in the context of prospective matched cohorts with predefined sampling times, can lead to significant ascertainment or sampling bias. In this regard, Sharma and colleagues' work shows an accumulation of both Teffs and Tregs in tumor samples following anti-CTLA-4 therapy, but the data does not fully preclude depletion of Tregs shortly after therapy with a subsequent enrichment of cases in whom this has either not occurred or has only

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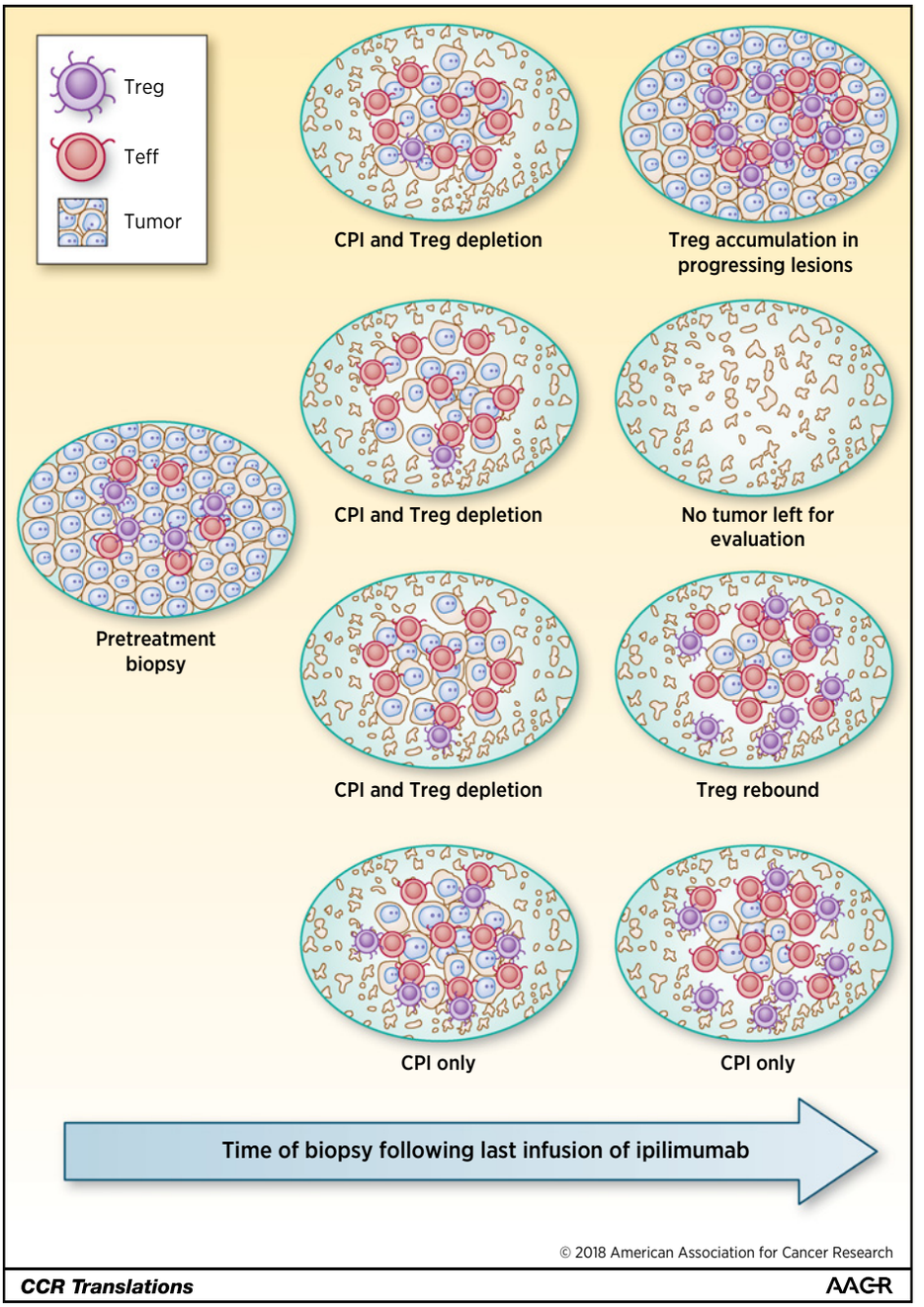

Figure 1.
Challenges deciphering the mechanism of action of anti-CTLA-4 in the clinic. Sample collection is one of the key challenges when translating murine mechanistic–based findings to the clinic. While in murine models, samples are collected shortly (days) after anti-CTLA-4 administration, in the clinic, sample collection can take weeks. This figure depicts several scenarios illustrating the checkpoint inhibiting (CPI) and potential Treg-depleting activity of anti-CTLA-4 antibodies. While biopsies taken shortly after therapy would be able to distinguish between the CPI and Treg-depleting activity of anti-CTLA-4, later sampling could be confounded by factors such as lesion progression (due to Treg accumulation) or rebound in numbers of Tregs posttherapy.

transiently occurred, by virtue of performing biopsies only in those with residual or progressive lesions (Fig. 1).

The issue is most prominent in the context of ipilimumab data. Ipilimumab bears the classical "depleting" human IgG1 backbone (like rituximab for example) and would be expected to demonstrate some depleting activity if this were critical to clinical activity. The comparison between naïve and treated patients, however, is complicated primarily by the timing of biopsies and potential sampling bias. The median time for sample collection was 8 and 18 weeks (prostate/bladder and melanoma, respectively) post-ipilimumab, with over half of the biopsies obtained more than 15 weeks after the last infusion. This contrasts starkly with mouse studies, where Treg depletion is evaluated shortly (3–10 days in most studies) after the last infusion of anti-CTLA-4. This point is critical, as after depletion by antibodies, chemo-, or radiotherapy, the remaining Tregs rapidly enter cell cycle and proliferate to replenish their empty niche. The rebound on Treg numbers observed in many mouse models postdepletion could help explain the high density of tumor-infiltrating Tregs observed by Sharma and colleagues weeks after the last administration of ipilimumab, particularly as this rebound will be most noticeable in nonresponding or progressing lesions. Whether biopsies in

untreated or treated patients take place in residual responding, stable, or progressing lesions as opposed to serial biopsies in a responding lesion may also significantly impact both findings and conclusions. One might hypothesize that evidence for Treg depletion may only be present in responding lesions and be maximal in lesions that completely regress, particularly as these antibodies have not been optimized for depleting activity. Response and depletion should also cosegregate with Fc polymorphism status (8). Furthermore, murine studies suggest that activity against both effector and regulatory compartments is important for optimal response. Activity limited to the effector compartment alone does result in antitumor response in murine models, while activity confined to the regulatory compartment alone does not. It is the combination that proves optimal (4). Depending on sampling criteria in the clinical data, one could be comparing mechanisms underpinning resistance rather than those defining response to therapy where residual or progressing lesions may well have more Treg than average or responding lesions. Of relevance, while prior studies by Ribas and colleagues did show Treg expansion in both progressing and regressing lesions, those studies were performed in the context of tremelimumab, a human IgG2 anti-CTLA4 with no predicted antibody-dependent cell-mediated cytotoxicity (ADCC) activity due to its poor binding to the activating human CD16 FcγR. It is unfortunate, then, that the data on serial evaluation of Treg numbers, which should have given more compelling evidence for the presence or absence of depleting activity, comes from patients treated with tremelimumab rather than ipilimumab.

Despite all of the caveats noted above, the data from Sharma and colleagues does argue against Treg depletion as a mechanism of action of ipilimumab and this needs to be further considered in the design of new trials. Recent work linking the clinical activity of ipilimumab to the presence of high-affinity FcR polymorphisms does suggest that anti-CTLA-4 antibodies require FcRs (and potentially Treg depletion) to drive maximal activity (8). However, these data also suggest that ipilimumab is a weak depleting agent as it requires a high-affinity FcR polymorphism for its maximal activity, potentially also contributing to the lack of depletion observed in Sharma's study.

Taken together, the mouse and human data still support the development of a next-generation of anti-CTLA-4 antibodies with the hypothesis that enhancing ADCC (and Treg depletion) will drive more potent and durable responses. It is also important, however, to consider the potential side effects associated with ADCC-enhanced anti-CTLA-4 antibodies. If ipilimumab's main mechanism of action does not involve Treg depletion, then systemic toxicities observed to date are likely driven by the checkpoint blocking activity of the antibody on Teff rather than by Treg depletion. In this case, next-generation anti-CTLA-4 antibodies with enhanced ADCC are likely to amplify toxicity. Whether such combined toxicities can be managed and whether the clinical responses obtained from such a next-generation therapeutic outweigh any additional toxicity will need to be evaluated with extreme caution.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S.A. Quezada, K.S. Peggs
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