Evaluate Vantage

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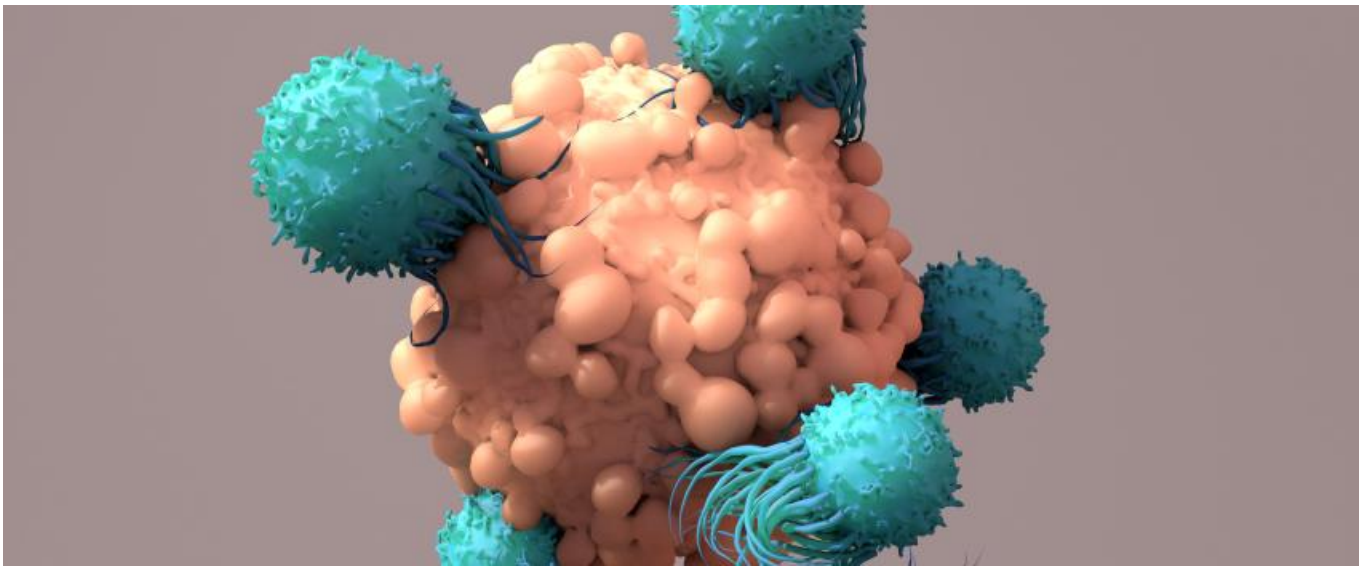
PD-1 versus Car-T: stop making sense

Paul Rennert



T-cell exhaustion, a frequently cited reason for Car-T relapse, might be reversed by PD-(L)1 blockade; but is exhaustion really the problem?

The 1984 film Stop Making Sense features the Talking Heads song "Crosseyed and Painless", which includes the staccato refrain:

I'm ready to leave

I push the fact in front of me

Facts lost

Facts are never what they seem to be

I thought of this lyric the other day when I read about a clinical study presented by researchers from Hospital Universitario de Salamanca at the recent EBMT-EHA Car-T cell meeting, entitled "Pembrolizumab after Car-T cell therapy: a single-centre experience".

The trial, in diffuse large B-cell lymphoma (DLBCL), was based on the premise that many initially responding patients relapse owing to T-cell exhaustion. PD-(L)1 blockade, such as Merck & Co's Keytruda, "is sometimes administered with the hope that it will reverse T-cell exhaustion following Car-T cell therapy", the authors wrote.

Exhaustion is quite the theme in immuno-oncology, and no fewer than 147 abstracts due to be presented at this weekend's AACR meeting mention it. But more of that later.

The Salamanca study considered 31 DLBCL patients who did not respond, or who responded but relapsed, after receiving Gilead's Yescarta or Novartis's Kymriah. 17 of these then got Keytruda every three weeks as salvage therapy, and four then saw complete remission, for an overall response rate of 23.5%.

I'd like to see more detailed data from this interesting study, as there are a few key items to consider here. First, the investigators report time since Car-T infusion, and the range was considerable, from a week and a half to seven months post-Car. Depending on that timing, and which Car product was administered, Car-T cellularity in patients will be markedly different at the different time points.

It would be useful to see a table that breaks out patients by Car product (Yescarta or Kymriah), time to first Keytruda infusion, and response. I'd also like to know the status of the normal B-cell and T-cell pools in these

patients.

Earlier work

The University of Pennsylvania separately published a similar study. 12 B-cell lymphoma patients who were either refractory to or relapsed after a CD19-directed, 4-1BB co-stimulated Car-T therapy (presumably based on Kymriah) were given Keytruda every three weeks. Time from Car-T cell infusion to first Keytruda dose ranged from 12 days to three and a half years, and the ORR was 25%, including one complete response.

The Penn team noted: “The optimal timing of [Keytruda] is a big question, and it appears that one year out is not optimal... Our study suggests that within three months is a better time frame, and we may be able to start much sooner or even before Car-T cell therapy. We treated some patients as early as 13 days after Car-T cell infusion without toxicity.”

But what in fact is going on with these patients?

The question is especially pertinent because we know from many failed studies that immune checkpoint therapy is not particularly efficacious in DLBCL. Keytruda is approved for primary mediastinal large B-cell lymphoma, and anti-PD-(L)1s are being studied front line in combination with standard of care. Still, progress with immune checkpoints here has been much slower than in solid tumours and in Hodgkin lymphoma, a distinct setting where Keytruda and Bristol Myers Squibb’s Opdivo are approved.

Note in the Salamanca study the statement that Keytruda “is sometimes administered with the hope that it will reverse T-cell exhaustion following Car-T cell therapy”. This is a broadly held assumption of how the Merck drug and other PD-(L)1 antagonists work, but in this instance I think the proposed mechanism is potentially misleading.

The PD-1 pathway

It helps to think about the role the PD-1 pathway plays in normal immune function to understand what is likely happening in the studies above, a subject extensively investigated, and reviewed, by many groups.

Put very simply, the interaction of PD-1 with PD-L1 damps down T-cell response, so the presence of PD-1 on T cells might correlate with their exhaustion. PD-(L)1 blockade can restore T cell function and, as the Salamanca and Penn trials suggest, some Car-relapsed patients can be rescued by giving them an anti-PD-(L)1 drug.

However, I don’t believe that there is a straightforward relationship between PD-1 and T-cell exhaustion. Indeed, nearly all subsets of activated T cells express PD-1; these are not all exhausted, and in fact most are fully functional. Rather, PD-1 acts as a rheostat, preventing unwanted T-cell activation.

PD-1 is indisputably not a marker of exhausted T cells, and in most settings it serves to modulate T-cell activation and mediate immune function without damaging normal cells, and without driving the T cell into a dysfunctional state of overactivation and exhaustion.

Exhaustion is a real phenomenon, of course. T cells appear to become functionally exhausted in the setting of chronic antigen presentation, as in chronic viraemia and in some solid tumour microenvironments. But it is not clear that this happens to Car-T cells in lymphoma patients, as both target cells and Car-T cells are rapidly turning over. Crucially, biomarker analyses have never identified the PD-1 pathway as a mediator of Car-T response in lymphoma, suggesting that a more complex cellular interplay is taking place.

Indeed, one can conceive of cell therapy as a frantic race between the proliferating cancer cells and Car-T cells, both placed under intense selective pressure. The question then is: if PD-1 activity is not hampering the Car-T response to lymphoma by inducing exhaustion, what is happening?

Does it have to be exhaustion?

We now know, from analyses performed by Stanford, the Moffitt Cancer Center, Kite/Gilead and others, that CD19 antigen density controls Car-T response in lymphoma in most patients. Moffitt and Gilead showed last year that average CD19 “brightness” on lymphoma cells was the single predictor of the effectiveness of Yescarta in the 2nd-line Zuma-7 DLBCL trial.

But what does CD19 antigen density have to do with response to anti-PD-(L)1 treatment after Car relapse? Do we need to invoke “exhaustion” as the de facto explanation for the observation that anti-PD-(L)1 treatment can reactivate Car-T cells in some patients?

Let’s consider the molecular consequences of signalling through the Car domain on the one hand, and through PD-1 on the other.

Car engagement triggers phosphorylation of the CD3z and co-stimulatory (CD28 or 4-1BB) domains and, as noted above, a minimum number of contacts between the CD19 protein and the anti-CD19 Car domain is needed to trigger Car-T cell activation, proliferation and effector functions.

While details differ between 4-1BB and CD28-based Cars, features like Lck activation and Zap70 phosphorylation and the subsequent formation of productive T-cell signalling complexes are roughly conserved. Activation of PI3K and its signalling cascade is mediated directly (CD28-based Car) or indirectly (4-1BB), though details are poorly understood. As with natural T-cell receptor (TCR) signalling, binding strength and duration are key variables.

PD-1 signalling requires prior T-cell activation, and is particularly enhanced when CD28 is activated. Control over this system is mediated by recruitment and activation of Lck by T-cell activation signals; Lck phosphorylates downstream signalling proteins as well as PD-1. PD-1 phosphorylation drives recruitment of SHP phosphatases, which counter the activation signals.

Simplistically, the integration of positive signals from the Car domains and negative signals from PD-1 occurs at the level of Lck and its components. These pathways are capable of further counter-regulation, as reduction in TCR or Car signalling reduces PD-1 activity and vice versa.

Car-T cell response

This model produces a prediction. Namely, that the strength of signalling through these two pathways (Car versus PD-1) controls Car-T cell responses.

The prediction is supported by published analyses finding that PD-1 signalling dramatically shifts the TCR/antigen binding dose-response curve, making T cells much less sensitive to TCR-generated signals. Too much or too little TCR signalling are both associated with dysfunction; PD-1's "rheostat" role is to keep T cells at an activation state that is "just right". This same dynamic appears to be in play in the context of Car-T signalling, with too much PD-1 signalling occurring with low antigen expression.

In B-cell lymphoma a second resistance mechanism drives this point home. The TCR complex and Car-T cell membrane include CD2, which binds to CD58 (aka LFA3) on various cell types including B cells. The CD2/CD58 pathway is not a major mediator of T cell activation except in settings of low antigen density.

Recent studies of responsiveness to Car-T cell therapy in lymphoma have highlighted loss or downregulation of CD58 expression as a mechanism of tumour cell escape. Since the antigen sensitivity of Car domains is less than that of native TCRs, this route of escape may be deployed in the lymphoma cell population to restrict antigen recognition further.

What's coming at AACR?

As ever, AACR is a haven of information on nascent mechanistic ideas in oncology. The most obvious fit with the theme above would be Cargo Therapeutics, which is developing Cars that overexpress CD2 to overcome resistance mechanisms, but it does not seem to be presenting at AACR.

What we will see is in vivo data from Evolveimmune Therapeutics, which has engineered a "CD2 agonist" into a CD3-based T-cell engager. A team from University of Colorado has encoded a Lat domain in a Car – Lat is an accessory enzyme in the Lck-Zap70 signalling cascade – and shows improved responses against low-antigen density tumour cells.

Investigators from Crystal Mackall's lab at Stanford, in a project supported by the Parker Institute and Kite/Gilead, will talk about protein-protein interactions that support Car-T efficacy.

And I must declare an interest too: Aleta is developing ALETA-001, a three-domain biological that contains the CD19 extracellular domain and binders against CD20 and albumin. The aim is to make lymphoma cells CD19-bright, thus preventing antigen-loss relapse; a clinical trial in patients previously treated with anti-CD19 Car-T should start mid-year.

The model I suggest above provides a simple framework for understanding the mechanisms of resistance to and relapse from anti-CD19 Car-T therapy. As noted previously, Mazner & Mackall analysed CD19 downregulation in their anti-CD19 Car-T cell-treated cohort at Stanford, and identified loss or downregulation of CD19 in 63% of their relapsed patients.

Rhein Shen of Kite/Gilead presented data at the IO Summit in Boston showing that the single predictor of lymphoma patient response to anti-CD19 Car-T cell treatment was CD19 brightness on the lymphoma cell population. These observations, and recent CD58-downregulation data, suggest that overall density of antigen/Car interaction determines outcome for most patients.

Some patients can be "pushed back" to productive Car signalling by blocking PD-1, thereby changing the balance of positive (Car) and negative (PD-1) signalling cascades. But there is no need to invoke "exhaustion" in the context of this model. "Facts are never what they seem to be."

Paul Rennert is chief executive and chief science officer of Aleta Biotherapeutics. He has no other financial or non-financial conflicts of interest, including with any of the other companies or organisations mentioned in this article.

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Evaluate HQ
44-(0)20-7377-0800

Evaluate Americas
+1-617-573-9450

Evaluate APAC
+81-(0)80-1164-4754