Evaluate Vantage

October 08, 2021

Allogene raises the spectre of a Car-T nightmare

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Little is known about yesterday's clinical hold on Allogene's entire pipeline, but chromosomal abnormalities hint at a worst-case scenario.

Clinical holds have recently shaken the gene therapy field, and now it's Car-T's turn. Allogene's revelation that chromosomal abnormalities were detected in the allogeneic Car-T cells infused into a lymphoma patient raises the possibility that gene editing in manufacturing the cells could disrupt their genome and make them capable of causing serious damage.

This, it has to be stressed, is an extremely bearish view, and more work is needed to determine what happened. But the risk to Allogene – off 40% this morning – and other allogeneic players is real. With Allogene's entire clinical pipeline on hold *Vantage Analysis* considers 10 key questions that must now be answered.

1. What exactly happened?

A lymphoma patient in the Alpha-2 trial of the CD19-directed allogeneic Car-T project ALLO-501A was found to have ALLO-501A cells containing a "chromosomal abnormality ... of unclear clinical significance". As a result the FDA has put on hold clinical trials of all Allogene projects.

ALLO-501A, like all of Allogene's allogeneic Car-T therapies, is based on T cells derived from healthy donors. These cells are then transduced with a lentiviral transgene coding for the Car, electroporated with Talen nucleases to knock out endogenous T-cell receptors, expanded and stored, before infusion into a patient.

2. When did the chromosomal changes occur?

If it was the gene editing that caused the problem the abnormality might have been detected just after manufacturing, but Allogene says all lots passed release tests, and showed no abnormalities. More precise testing, including periodic draws done during the patient's treatment, should elucidate the timeframe further.

3. Why was the issue not detected after manufacturing?

Every quality control assay has its sensitivity limits, and if the abnormality was present in just a handful of cells – or even just a single cell – it could conceivably have evaded detection. The prospect of an abnormal cell undergoing clonal expansion is, however, bad.

4. Could it have occurred after cell infusion?

Allogene says rapid T-cell expansion, which typically happens when antigen targets are encountered, can cause changes in those cells, including genetic mutations, deletions or inversions. This scenario would absolve gene editing of blame, and Allogene bulls will cling to this hope.

5. How close is the abormality to where Allogene's Talens cut?

This is perhaps the $64,000 Question. Among the most damningly suggestive of Allogene's revelations was that the abnormality occurred on chromosome 14.

Chromosome 14 happens to contain the Trac locus, which is what Allogene's Talen nucleases knock out to prevent expression of endogenous T-cell receptors. The bears will argue that this suggests that Talen gene editing brought about the chromosomal change.

However, Allogene says it does not have enough information on where precisely on chromosome 14 the changes occurred. As the human genome has 23 chromosomes the chances of the abnormality happening on chromosome 14 are one in 23.

A separate point is that ALLO-501A undergoes two rounds of gene editing – to knock out T-cell receptor as well as CD52 expression. Other gene editing methods have suggested that multiple edits increase the chances of chromosomal translocation.

6. So can we rule out lentiviral integration problems?

It is still possible that the chromosomal disruption is a result of lentivirus integration, but this needs to be investigated further. Insertional mutagenesis was a major talking point at Kymriah's 2017 US adcom, but was ultimately dismissed.

However, it remains a theoretical concern. Should Allogene show a risk the effect could be felt beyond allogeneic Car-T players, in the autologous lentiviral space.

7. Why were not all the ALLO-501A cells affected?

Allogene revealed that only a "fraction" of the ALLO-501A cells in the patient were affected by the abnormality, and has yet to investigate the kinetics of the clone in question. On the positive side the deleterious clone had not become dominant in this patient, though such a scenario cannot be ruled out in future.

8. Could this problem actually be quite common?

The abnormality might not have been picked up at all had the patient not undergone bone marrow biopsy to investigate progressive pancytopenia. Allogene says it has treated over 100 patients with its allogeneic Car-T therapies without seeing something similar, but bone marrow biopsy is not done as standard.

9. Are there confounding factors?

The patient had transformed follicular lymphoma and c-Myc rearrangement, had got chemo/radiotherapy and steroids, and was to have received autologous Car-T therapy but their cells failed to expand adequately. In addition they underwent allogeneic stem cell transplant after reporting a partial response to ALLO-501A.

Some of these might have contributed, and whether the influence of each can be ruled out definitively remains a big problem.

10. If this is an editing problem, is it Talen-specific?

At present the big market casualties are Allogene, its licensor and Talen nuclease specialist Cellectis (off 17%) and its future electroporation partner Maxcyte (-11%).

Though publications have cited chromosomal deletions, translocations and inversions with certain other gene editing methods, the Crispr-focused allogeneic Car-T players Poseida and Crispr Therapeutics, the Arcus nuclease specialist Precision Biosciences and the zinc-finger editing company Sangamo are this morning trading flat. But Allogene's problem could signal a broad issue with all gene editing, and not just with Talens.

It is true that Car-T has weathered toxicity problems, notably with cytokine storm and cerebral oedema, and Allogene's current management is notable for having steered Kite Pharma through such a storm. For now, however, all Allogene clinical work is suspended, and the latest problem has the potential to be orders of magnitude more serious.

On hold: Allogene's clinical pipeline		
Trial	**Project**	**Indication**
Alpha	ALLO-501 (anti-CD19 with Rituxan switch)	R/r non-Hodgkin lymphoma
Alpha-2	ALLO-501A (anti-CD19 without Rituxan switch)	R/r non-Hodgkin lymphoma
Universal	ALLO-715 (anti-BCMA)	R/r multiple myeloma
Traverse	ALLO-316 (anti-CD70)	Renal cell carcinoma
Ignite	ALLO-605 (anti-BCMA with chimaeric cytokine receptor)	R/r multiple myeloma
Source: company website & clinicaltrials.gov.		