**CYAD-101: An innovative non-gene edited allogeneic CAR-T for solid tumor cancer therapy**

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**BACKGROUND**

- Allergic chimeric antigen receptor (CAR)-T cells are produced from healthy donor cells, collected and banked, and then activated, before becoming CAR-T cells, essentially the same time delay achieved for production of the autologous product.
- While allergic CAR-T cell approaches have started to show some promise in hematological cancers in humans, to date, researchers have struggled to find similar success in more challenging solid tumor indications.

**CYAD-101 is a novel, first-in-class, allogenic NKG2D receptor-based CAR-T cell product**

- The CYAD-101 CAR-T cell product uses a non-gene edited T-cell receptor (TCR) idiotype molecule (Tm) to control graft-versus-host disease (GvHD), combined with CAR based on the CD16 ligand.
  - The NKG2D CAR comprises the full-length human NKG2D receptor fused to the intracellular signaling domain of DAP12.
  - NKG2D binds eight stress ligands that are over-expressed in a broad range of solid tumors including metastatic colorectal cancer (mCRC).
  - Tm is a truncated peptide that competes with the endogenous CD3ε in the TCR complex formation, thus reducing the ability of the endogenous TCR to activate, while not impacting the activity of the NKG2D CAR.

- The CYAD-101 treatment options available for mCRC patients who have refractory disease after the second line therapy, NKG2 patients with a lack of therapy have a median progression-free survival of 3 months.

- Clinical grade CYAD-101 cells were produced for the Phase 1 alloSHRINK study (NCT03692429) which evaluated the concurrent administration of CYAD-101 with BevCyt (anti-VEGF) and Pembrolizumab in melanoma patients. The use of FOLOX was primarily as a lymphodepletion regimen to facilitate CYAD-101 engraftment and avoid alloreactivity and also inducing an up-regulation of NKG2D expression on the cancer cells.

- FOLOX was not intended to induce a cytokotic effect on the cancer cells as most patients were refractory to previous treatment lines.

**METHODS**

- A bank of 13 billion clinical grade CYAD-101 cells was generated in two production runs, one using 10 million Tm-transduced CYAD-101 cells per patient and a second using 30 million Tm-transduced CYAD-101 cells per patient.
- The CYAD-101 product, generated from both production runs was highly enriched and consisted of 86% CD+ T cells with a transduction level of 90%, low relative expression of CD3ε/CD8 and minimal expression of CD25/49D.
- The CYAD-101 cells were predominantly T-Rex cluster of differentiation (CD16a/CD56/TCR-γ-δ) suggestive of an effector T-cell population.

- The Phase 1 alloSHRINK study:
  - Patient population.
  - Non-refractory mCRC patients with recurrent/progresing disease after at least one metastatic treatment line including oxaliplatin and irinotecan-based chemotherapy.
  - Primary objective.
  - Definition of the recommended dose based on the occurrence of dose-limiting toxicity (DLT) and overall safety as defined in the treatment phase.

- Study design.
  - Open-label, dose-escalation study with a 3:1 allocation ratio of patients to a 3-level (DL1: 1x10^8, DL2: 1.5x10^8, and DL3: 3x10^8 cells per injection) and small expansion segment with at least one patient at the recommended dose level.

- Treatment schedule.
  - Intravenous CYAD-101 injections at 2-week interval and at Day 3 of three consecutive FOLOX chemotherapy cycles (standard doses).

**TABLES & FIGURES**

**Table 1: Clinical outcomes**

- **Best overall responses**
  - The target disease was mCRC.
  - 4 lines of therapy have a median progression-free survival of 3 months.
  - A total of 15 mCRC patients have been treated in this Phase I alloSHRINK study (3 at DL 1, 3 at DL2 and 9 at DL3) (Table 2).
  - The proportion of patients with any related AE (%) was 7 (46.7%) out of 15 patients.
  - Patients with any related AE (%) 7 (46.7%) out of 15 patients.
  - Overall incidence of AEs: 183 (100%) out of 15 patients.
  - Overall safety and clinical activity data are HLA-independent indicating that CYAD-101 cells can be used in a broad population regardless of the HLA haplotype.

- **Table 2: Incidence of adverse events (AEs)**
  - Out of 15 patients, 2 patients with PR confirmed by RECIST 1.1 criteria and 6 patients with SD (100%) achieved partial remission.
  - Overall treatment-related AEs: 183 (100%) out of 15 patients.
  - Overall incidence of AEs: 183 (100%) out of 15 patients.

**Figure 1: CYAD-101 activity in mCRC**

- The CYAD-101 CAR-T cell product was used in the first clinical study to test the non-gene edited CYAD-101 CAR-T cell therapy in a solid cancer indication.

**Figure 2: Flow cytometric validation of the CYAD-101 clinical run**

- CYAD-101 failed to induce significant levels of GvHD upon intravenous injection in irradiated NSG mice, in contrast to control T-cells.

**Figure 3: Time to response and duration of treatment**

- The CYAD-101 cells were detected in the peripheral blood by polymerase chain reaction-based methods.

**Figure 4: CYAD-101 kinetics in the peripheral blood (peak engraftment Cmax)**

- Upon co-culture with target SKEL cells, CYAD-101 readily produced IFN-γ that was blocked by a NKG2D inhibitory antibody.

**CONCLUSIONS**

- CYAD-101 is a novel, first-in-class, allogenic NKG2D receptor-based CAR-T cell therapy.

- To our knowledge, the alloSHRINK study (NCT03692429) is the first clinical study that used a non-gene edited allogeneic CAR-T cell therapy in a solid cancer indication.

- CYAD-101 demonstrated a favorable safety profile at all evaluated dose-levels, with no Grade 5 toxicity observed.

- The dose of 1x10^8 CYAD-101 cells per injection post-FOLOX chemotherapy is defined as the recommended dose for further development.

- The response rate of two patients with confirmed PR, including one KRAS mutated patient, out of 15 patients is an encouraging indicator of clinical activity in mCRC patients.

- No evident correlation could be observed between disease control and patient baseline characteristics.

- The drop in CR studied observed after the 2nd and 3rd CYAD-101 injections is possibly the result of a more rapid clearance of CAR-T cells. Despite use of FOLOX as a lymphodepletion regimen, the development of a host versus graft reaction cannot be ruled out and warrants further investigations.

- Overall safety and clinical activity data are HLA-independent indicating that CYAD-101 cells can be used in a broad population regardless of the HLA haplotype.

- These early clinical results demonstrating the safety of a non-gene edited allogeneic CAR-T cell approach with no signs of graft versus host disease together with the closedactivity observed in incurable mCRC patients support the further development of the CYAD-101 allogeneic CAR-T cell technology.

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**Any questions? Please contact us at contact@celadon.com**