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LETTER TO THE EDITORS

CAR T cells targeting solid tumors: carcinoembryonic antigen (CEA) proves to be a safe target

Astrid Holzinger[1,2] · Hinrich Abken[1,2]

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Dear Editors,

Two recently published trials (NCT01212887 and NCT02349724) provided some clinical efficacy in the treatment of gastrointestinal adenocarcinoma by systemic application of chimeric antigen receptor (CAR) engineered T cells redirected against carcinoembryonic antigen (CEA) [1, 2]; local administration of anti-CEA CAR T cells by hepatic artery infusion also decreased tumor progression (NCT01373047) [3] (Table 1).

While CAR T cell therapy is inducing lasting remissions and even cure in the treatment of hematologic malignancies, the treatment of solid tumors still remains challenging; CAR T cell toxicities to healthy tissues have frequently required trial cessation. The recent anti-CEA CAR T cell trials for the first time demonstrate that targeting an autoantigen, which is physiologically expressed by the luminal epithelia of the gastrointestinal tract and the lung, is feasible without severe tissue destruction. Targeting CEA has the advantage that healthy cells expose CEA in a polarized fashion on the luminal side, while cancer cells express the antigen over the entire cell surface. As a consequence, CEA on cancer cells is recognized by CAR T cells which become activated and finally eliminate the targeted cancer cells, while healthy cells with luminal CEA remain invisible to CAR T cells. However, the polarized CEA distribution collapses upon tissue injury in micro-lesions under physiologic conditions; as long as the lesion remains small, anti-CEA CAR T cell activation resulting in clinically relevant inflammation is less likely. T cell targeting by a TCR, in contrast to CARs, faces a different challenge; presented by the HLA present on the entire cell surface, CEA is recognized by TCR engineered T cells on healthy cells as well, with the risk of inducing auto-immune toxicity; indeed, a previous trial with TCR engineered T cells resulted in such toxicities which were severe and dose-limiting [4]. Thus, anti-CEA CAR T cells have an advantage over TCR engineered T cells in this context.

Moreover, there are some differences in the clinical protocols for using these agents. First, the CAR binding domains target different epitopes of the extracellular CEA moiety; the BW431/26 scFv CAR targets the A3B3 domain which is close to the cancer cell membrane, whereas the MFE23 scFv CAR binds to the far distal N-A1 domain. Targeting the membrane-proximal CEA epitope is more efficient for CAR T cell activation than targeting the distal epitope independently of the binding affinity. Second, T cells with a first-generation CD3ζ CAR (NCT01212887) persisted poorly, in contrast to T cells with a second-generation CD28–CD3ζ CAR (NCT02349724). CAR T cell persistence and amplification is a strong predictor of clinical efficacy which cannot be fully compensated by high IL-2 doses as applied in the CD3ζ CAR trial; no IL-2 was administered in the CD28–CD3ζ CAR trial. Systemic IL-2 support also improved the anti-tumor responses of locally applied CAR T cells (NCT01373047), but this was at the cost of more severe adverse events. CAR T cell amplification is likewise promoted by "pre-conditioning", a non-myeloablative lymphodepletion procedure immediately before CAR T cell administration to provide space,

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✉ Hinrich Abken
hinrich.abken@uk-koeln.de

[1] Center for Molecular Medicine Cologne, University of Cologne, Robert-Koch-Str. 21, 50931 Cologne, Germany

[2] Clinic I Internal Medicine, University Hospital Cologne, Robert-Koch-Str. 21, 50931 Cologne, Germany

Table 1 Adoptive therapy trials with anti-CEA CAR T cells

	NCT02349724 PI: Cheng Qian	NCT01212887 PI: Robert E. Hawkins	NCT01373047 PI: Steven C. Katz
CAR	BW431/26 scFv-IgG4 hinge-CD28–CD3ζ; humanized scFv	MFE23 scFv-CD3ζ; murine scFv	MN14 scFv-CD8hinge-CD28–CD3ζ; humanized scFv
Antibody affinity	10^{-10} M	2.5×10^{-9} M	10^{-9} M
Vector	Lentivirus	γ-retrovirus	γ-retrovirus
Patient cohorts	10 relapsed and refractory, metastatic colo-rectal cancer patients; 7 × M, 3 × F; median age 58 years	14 relapsed and refractory, metastatic gastrointestinal cancer patients; 8 × M, 6 × F; median age 48 years	6 patients with liver metastases of adenocarcinoma (eight patients enrolled); 4 × M, 2 × F; average age 57 years
Pre-conditioning	FLU (25 mg/m^2 for 2 days); CTX (300 or 900 mg/m^2 for 3 days)	Cohort 1-3: FLU (25 mg/m^2/day for 5 days); cohort 4: FLU (25 mg/m^2/day for 5 days) and CTX (60 mg/kg/day for 2 days)	None
Administration	Systemic infusion	Systemic infusion	Percutaneous hepatic artery infusion
T cell dose	Escalating doses: 1×10^5–1×10^8/CAR$^+$ T cells/kg (2.5×10^7–1.5×10^{10} total T cells); split doses (10, 30, and 60% of the total cell dose)	Escalating doses: $0.21–3.89 \times 10^9$ total dose of CAR T cells (10^9–5×10^{10} total T cells)	First cohort: escalating doses: 10^8, 10^9, and 10^{10} CAR T cells; Second cohort: 3 injections with 10^{10} CAR T cells plus systemic IL-2 support
IL-2 administration	None	600,000 IU/kg per dose; 2–12 intravenous bolus infusions	75,000 U/kg/day
T cell engraftment	CAR T cell engraftment and proliferation especially after a second CAR T cell therapy	Short-lived CAR T cell engraftment with a rapid decline within 14 days	CAR T cells transiently detected in the peripheral blood in 2/6 patients
Best tumor response	7/10 patients with stable disease	7/14 patients with stable disease	1/6 patient with stable disease
	Decline of serum CEA levels	Transient reduction in serum CEA in cohort 4	Decline of serum CEA levels
	No colitis even at high-dose CAR T cell infusion	No colitis	Necrosis or fibrosis of liver metastases in 4/6 patients
	No respiratory toxicity	Transient, acute respiratory toxicity	

CTX cyclophosphamide, *F* female, *FLU* fludarabine, *M* male, *PI* principal investigator, *scFv* single chain fragment of variable region

cytokines, and other growth factors. The optimal pre-conditioning regimen is still a matter being explored, balancing the risk of side effects versus the capacity to sustain T cell amplification. In conjunction with IL-2 administration, in particular, pre-conditioning is a major cause of severe adverse events.

The CD3ζ CAR trial (NCT01212887) was set on hold, because patients suffered from shortness of breath, although this was transient and clinically manageable. Pulmonary dysfunction frequently occurs independently of the T cell specificity and is thought to be due to the accumulation of activated T cells in the lung capillaries immediately after infusion, often requiring artificial respiration. As long as the lung epithelia layer is not largely disrupted due to secondary events, it is unlikely that the anti-CEA CAR T cells are directly causing pulmonary damage.

As the risk of toxicities increases, defining early surrogate markers for response will be crucial to identify those patients who will benefit from treatment. Serum IFN-γ as an indicator of T cell activation will not be suitable, because IL-2 supplementation increases IFN-γ independently of CAR T cell activation. A specific marker in this situation is the CEA level in serum which is routinely recorded to monitor tumor load. Indeed, CEA levels declined in patients receiving the highest CAR T cell dose in all three trials, strongly supporting the notion that the anti-CEA CAR T cells were mediating a productive anti-tumor response. Serum CEA does not induce or interfere with CAR T cell activation, because it does not cluster the CAR molecules required to form a signaling synapse.

The results of these trials together support our assertion that CEA as an auto-antigen with a strictly luminal expression pattern can be safely targeted by CAR T cells, resulting in some therapeutic efficacy in the treatment of solid tumors.

Astrid Holzinger
Hinrich Abken

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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