Hypophosphatemia is Associated with Neurotoxicity Symptoms in CAR-T Cell Therapy

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Abstract

Adoptive immune cell therapies, including genetically engineered chimeric antigen receptor (CAR)-T cell therapy have greatly transformed the landscape of cancer treatment for several cancers, including relapsed/refractory non-Hodgkin's lymphoma, diffuse large B cell lymphoma, and acute lymphoid leukemia [1]. While effective, CAR-T cell therapies can be limited by neurological cytokine release syndrome (N-CRS), which is characterized by confusion, dysphasia/aphasia, impaired fine motor skills, somnolence, and in more severe cases, seizures, motor weakness, cerebral edema, and coma [8]. N-CRS is currently managed through supportive care and high-dose corticosteroids. The use of corticosteroids is not ideal, given that the immunosuppression can potentially attenuate the anti-tumor activity of the treatment itself [8]. Previous studies dealing with refeeding syndrome, which can occur when administering nutrition to malnourished individuals, have demonstrated rapid evolution of hypophosphatemia due to an iatrogenic energetic crisis. The rapid introduction of carbohydrates causes an insulin surge that promotes rapid cellular uptake and use of glucose and phosphate, leading to extreme depletion of serum phosphorus, and onset of neuromuscular and psychiatric symptoms comparable to those seen in CAR-T patients experiencing N-CRS [2]. The goal of my project is to investigate the relationship between CAR-T cell neurotoxicity and serum phosphorus levels. I hypothesize that the increased metabolic demand of these highly activated genetically engineered immune cells is causing clinically significant hypophosphatemia, leading to neurotoxicity. If true, a safe, non-invasive serum phosphorus replenishment protocol can be introduced to prevent or reduce the severity of CAR T therapy-induced neurotoxicity.

Methods

1 Retrospective chart review for 77 patients at UCLA with B cell malignancy (DLBCL, NHL, ALL) treated with Yescarta or Kymriah CD19-targeting CAR T cell therapy

1. Neurological symptoms, ICANS (immune effector cell-associated neurotoxicity syndrome) score, CARTOX-10 score, and blood chemistry were obtained from CareConnect for each patient from day of CAR T cell infusion to day of discharge
2. Correlation between serum phosphorus and ICANS score was calculated for the entire cohort and for individual patients
3. Linear mixed effect model was generated in R based on the cohort with neurotoxicity, taking into account serum phosphorus, ICANS score, CARTOX-10 score, and time

2 In vitro co-culture assay to explore a mechanism of serum phosphorus depletion by CAR T cell therapy

1. CD19-targeting human CAR T cells with either CD28-CD3z or 41BB-CD3z signaling domains and non-CD19-targeting "mock" CAR T cells were co-cultured at varying E:T ratios with GFP-FLuc-Raji B cells for 24, 36, 48, and 72 hours
2. Tumor cell killing was assessed using a firefly luciferase glow assay
3. Colorimetric assays were used to assess changes in media phosphate and glucose

Results

ICANS
Aphasia
Altered Consciousness
Impaired Cognition
Motor Weakness
Seizure
Cerebral Edema

Table 6
ASTCT ICANS Consensus Grading for Adults

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score*	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
Depressed level of consciousness†	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 min); or Repetitive clinical or electrical seizures without return to baseline in between
Motor findings‡	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Elevated ICP/ cerebral edema	N/A	N/A	Focal/local edema on neuroimaging§	Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad

ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause; for example, a patient with an ICE score of 3 who has a generalized seizure is classified as grade 3 ICANS.
N/A indicates not applicable.
* A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 ICANS if unarousable.
† Depressed level of consciousness should be attributable to no other cause (eg, no sedating medication).
‡ Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.
§ Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.

Table 1
Clinical signs and symptoms of refeeding syndrome

Hypophosphatemia	Hypokalemia	Hypomagnesemia	Vitamin/Thiamine Deficiency	Sodium Retention	Hyperglycemia
Cardiac Hypotension Decreased stroke volume Respiratory Impaired diaphragm contractility Dyspnea Respiratory failure Neurologic Paresthesia Weakness Confusion Disorientation Lethargy Ataxic paralysis Seizures Coma Hematologic Leukocyte dysfunction Hemolysis Thrombocytopenia Other Death	Cardiac Arrhythmias Respiratory Failure Neurologic Weakness Paralysis Gastrointestinal Nausea Vomiting Constipation Muscular Rhabdomyolysis Muscle necrosis Other Death	Cardiac Arrhythmias Neurologic Weakness Tremor Tetany Seizures Altered mental status Coma Gastrointestinal Nausea Vomiting Diarrhea Other Refractory hypokalemia and hypocalcemia Death	Encephalopathy Lactic acidosis Death	Fluid overload Pulmonary edema Cardiac compromise	Cardiac Hypotension Respiratory Hypercapnea Failure Other Ketoacidosis Coma Dehydration Impaired immune function

Data from Kraft MD, Btaiche IF, Sacks GS. Review of RFS. Nutr Clin Pract 2005;20:625–33.

Figure 1: (A) ASTCT guidelines for grading neurological toxicity associated with immune effector cells (ICANS scoring system) [2] and (B) Clinical signs and symptoms of refeeding syndrome [3]

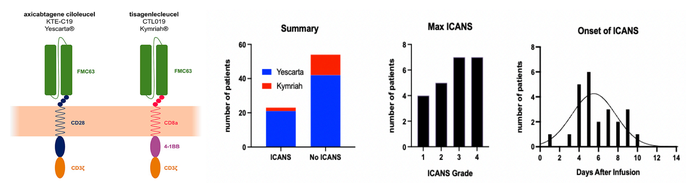

Figure 2: (A) Yescarta (CD28-CD3z signaling domain) vs Kymriah (4-1BB-CD3z signaling domain) [8] (B) 77 patients treated at UCLA (23 experienced ICANS - 21 *Yescarta*, 2 *Kymriah*) (C) Histogram showing max ICANS grade of each patient (C) Histogram showing earliest onset of ICANS of each patient with average time of onset at day 5 post-infusion

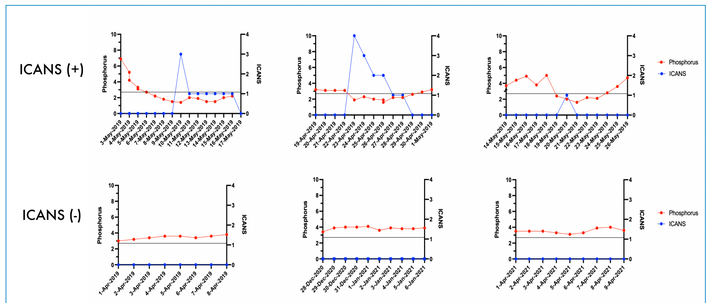

Figure 3: Representative patients with ICANS (top row) and without ICANS (bottom row)

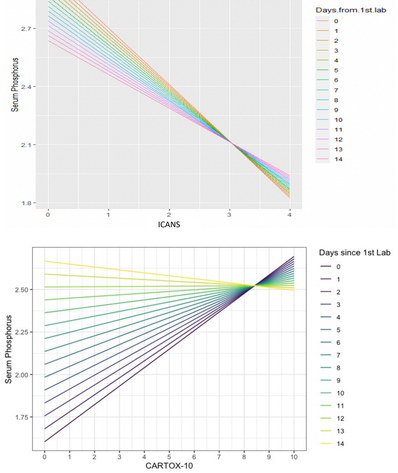

Figure 4: Linear mixed-effect model predicts a 0.29 decrease in serum phosphorus for every grade increment in ICANS ($p<0.0001$) and a 0.11 increase in serum phosphorus for each point of CARTOX-10 ($p=0.002$)

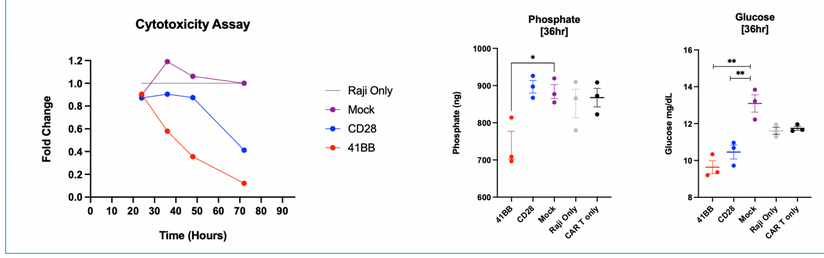

Figure 5: (A) Fold change in luminescence (relative to tumor alone) from 10:1 E:T ratio co-culture assay using CD19-41BB-CD3z, CD19-CD28-CD3z, and Mock scFv CAR T cells against GFP-Fluc-Raji B cells. (B) Media phosphate content from 10:1 CAR-T:Raji co-culture measured at 36 hours. (C) Media glucose content from 10:1 CAR-T:Raji co-culture measured at 36 hours.

Discussion

Toxicity from CAR-T cell therapy can be classified into two separate clinical syndromes both in onset and presentation: Cytokine Release Syndrome (CRS) and Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS). CRS presents earlier, with features of a systemic inflammatory response including fever, malaise, anorexia, hypotension, hypoxia, and widespread organ dysfunction. In contrast, ICANS generally occurs after CRS, presenting with confusion, dysphasia/aphasia, impaired fine motor skills, somnolence, and in more severe cases, seizures, motor weakness, cerebral edema, and coma [8].

The link between hypophosphatemia and neurotoxicity comes from the similar clinical presentation of refeeding syndrome, which occurs during nutritional rehabilitation of a malnourished patient. Hypophosphatemia is a hallmark of refeeding syndrome, as the rapid introduction of carbohydrates can cause an insulin surge that promotes rapid cellular uptake and use of glucose and phosphate, leading to extreme depletion of serum phosphorus. This presents as confusion, disorientation, somnolence, motor weakness, paresthesia, seizure, and coma [2]. Refeeding hypophosphatemia can occur within 24-72 hours of introducing nutrition, with a nadir during the first week, and according to several case reports, even small decreases in serum phosphorus can result in large-scale dysfunction when phosphorus levels are sufficiently low [10].

It is possible that in the setting of CAR-T cell therapy, by greatly increasing the metabolic demand for phosphorylated intermediates through the introduction of hundreds of millions of highly metabolically active, antigen-directed cells (2E6 CAR+ T cells/Kg body weight), hypophosphatemia can occur, leading to the neurological symptoms seen in refeeding syndrome [9].

Indeed, a retrospective chart review of 77 UCLA patients receiving anti-CD19 CAR-T cell therapy for B cell malignancies (DLBCL, NHL, or ALL) between 7/1/2018 – 5/1/2021 revealed a modest negative correlation between serum phosphorus and ICANS score, with a Spearman correlation coefficient of -0.23, $p<0.00001$. This approach, however, does not account for the temporal nature of the relationship, nor the range of serum phosphorus values when hypophosphatemia becomes clinically significant. Looking at the data from individual patients, neurotoxicity can occur days after serum phosphorus drops to clinically significant levels (Figure 4). A linear mixed effect model was generated to better depict this relationship. Here, the strength of the negative correlation between serum phosphorus and ICANS score and likewise, the positive correlation between serum phosphorus and CARTOX-10 score changes as a function of time. Moreover, an increase in ICANS and decrease in CARTOX-10 only occurs within a clinically significant range of serum phosphorus values (figure 5).

Next, in order to establish a putative mechanism for CAR T cell infusion driven serum hypophosphatemia, human CAR T cells with a CD19 targeting scFv driving either 4-1BB-CD3z or CD28-CD3z signaling and "mock" CAR T cells with a non-targeting scFv were co-cultured with CD19+ Raji B cells. Here, CAR T cell-induced tumor lysis, measured as a decline in Raji luciferase activity, corresponds with increased media phosphate and glucose consumption (figure 6).

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