Modified-EASIX predicts severe cytokine release syndrome and neurotoxicity after Chimeric Antigen Receptor (CAR) T cells

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Abstract:
Patients who develop CAR T-cells-related severe CRS and immune-effector-cells-associated neurotoxicity syndrome (ICANS) exhibit hemodynamic instability and endothelial activation. The EASIX (Endothelial-Activation and Stress-Index) score [LDH (U/L)×creatinine (mg/dL) / platelets (10^9 cells/L)] is a marker of endothelial damage that correlates with outcomes in allogeneic hematopoietic cell transplantation. Elevated LDH and low platelets have been associated with severe CRS and ICANS, as has C-reactive protein (CRP), while increased creatinine is seen only in a minority of advanced severe CRS cases. We hypothesized that EASIX and two new modified EASIX formulas [simplified-EASIX, which excludes creatinine, and modified-EASIX (m-EASIX), which replaces creatinine with CRP (mg/dL)], calculated peri CAR T-cells infusion, would be associated with development of severe (grade [greater than or equal to]3) CRS and ICANS. We included 118 adults, 53 with B-acute lymphoblastic leukemia treated with 1928z CAR T-cells (NCT01044069) and 65 with diffuse large B-cell lymphoma treated with axicabtagene-ciloleucel or tisagenlecleucel. The three formulas showed similar predictive power for severe CRS and ICANS. However, low platelets and high CRP values were the only variables individually correlated with these toxicities. Moreover, only m-EASIX was a significant predictor of disease response. m-EASIX could discriminate patients who subsequently developed severe CRS preceding the onset of severe symptoms (AUC: at lymphodepletion 80.4%, day -1 73.0%, day +1 75.4%). At day +3, it also had high discriminatory ability for severe ICANS (AUC 73%). We propose m-EASIX as a clinical tool to potentially guide individualized management of patients at higher risk for severe CAR T-cells-related toxicities.

Conflict of interest: COI declared - see note

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- m-EASIX calculated before and early after CAR T-cells infusion can predict severe CRS and ICANS before the onset of severe symptoms
- m-EASIX includes laboratory parameters routinely available in CAR T-cells clinical practice, and can be easily calculated at bedside

Abstract

Patients who develop CAR T-cells-related severe cytokine release syndrome (CRS) and immune-effector-cells-associated neurotoxicity syndrome (ICANS) exhibit hemodynamic instability and endothelial activation. The EASIX (Endothelial-Activation and Stress-Index) score [lactate dehydrogenase (LDH, U/L) × creatinine (mg/dL) / platelets (10^9 cells/L)] is a marker of endothelial damage that correlates with outcomes in allogeneic hematopoietic cell transplantation. Elevated LDH and low platelets have been associated with severe CRS and ICANS, as has C-reactive protein (CRP), while increased creatinine is seen only in a minority of advanced severe CRS cases.

We hypothesized that EASIX and two new modified EASIX formulas [simplified-EASIX, which excludes creatinine, and modified-EASIX (m-EASIX), which replaces creatinine with CRP (mg/dL)], calculated peri CAR T-cells infusion, would be associated with development of severe (grade ≥3) CRS and ICANS. We included 118 adults, 53 with B-acute lymphoblastic leukemia treated with 1928z CAR T-cells (NCT01044069) and 65 with diffuse large B-cell lymphoma treated with axicabtagene-ciloleucel or tisagenlecleucel. The three formulas showed similar predictive power for severe CRS and ICANS. However, low platelets and high CRP values were the only variables individually correlated with these toxicities. Moreover, only m-EASIX was a significant predictor of disease response. m-EASIX could discriminate patients who subsequently developed severe CRS preceding the onset of severe symptoms (AUC: at lymphodepletion 80.4%, day -1 73.0%, day +1 75.4%). At day +3, it also had high discriminatory ability for severe ICANS (AUC 73%). We propose m-EASIX as a clinical tool to potentially guide individualized management of patients at higher risk for severe CAR T-cells-related toxicities.
Introduction

The Endothelial Activation and Stress Index (EASIX) score [Lactic Dehydrogenase (LDH, U/L) x Creatinine (mg/dl) / platelets (PLT, 10^9 cells/L)] has emerged as a marker of endothelial damage and predictor of survival in patients with acute graft-versus-host-disease (GVHD) after allogeneic hematopoietic cell transplant (allo-HCT).\(^1\) EASIX assessed pre-HCT has also been shown to predict significant fluid overload after transplant, increased risk of acute GVHD, and to be associated with non-relapse mortality (NRM) and overall survival (OS) after allo-HCT.\(^2\)–\(^5\)

Patients who develop Chimeric Antigen Receptor (CAR) T-cells-related severe cytokine release syndrome (CRS) and immune-effector-cells-associated neurotoxicity syndrome (ICANS) exhibit hemodynamic instability and coagulopathy, with evidence of endothelial activation and increased blood brain barrier permeability.\(^6\) Several inflammatory cytokines and markers of coagulopathy have been shown to correlate with the onset of severe CRS and/or ICANS, such as Interferon-\(\gamma\), Interleukin-6 (IL-6), IL-1, IL-10, monocyte chemoattractant protein-1 (MCP-1), and angiotensin-2 (Ang-2).\(^6\)–\(^9\) Among routinely available laboratory tests, LDH, CRP, fibrinogen, platelets, and ferritin have been associated with severe CRS and severe neurotoxicity.\(^6,\)\(^7,\)\(^10\)–\(^13\)

We hypothesized that the EASIX score, as a marker for endothelial damage, would be able to predict the onset of severe CRS and/or ICANS in patients receiving CAR T-cells. Creatinine, one of the components of EASIX, however, plays a limited role as a biomarker of CAR T-cell-related toxicities, with elevation in a minority of patients who have already developed severe symptoms of CRS.\(^14\) Therefore, we further hypothesized that two modified EASIX formulas, the simplified-EASIX (s-EASIX), which excludes creatinine, and the modified-EASIX (m-EASIX), which replaces creatinine with CRP (mg/dL), would better estimate the risk of developing severe CRS and ICANS after CAR T-cells infusion. Therefore, we calculated EASIX/s-EASIX/m-EASIX scores at different timepoints pre- and early post-infusion in recipients of CAR T-cells in order to investigate their association with CRS and ICANS.

Methods

**Study Population and Data Collection**

This retrospective analysis included adult patients with B-cell acute lymphoblastic leukemia (B-ALL) enrolled at our center in a phase I trial of 1928z CAR T-cells from 2010 through 2016 (#NCT01044069),\(^12\) and consecutive patients with Non-Hodgkin Large B cell lymphoma (LBCL)
treated at our center with commercially available axicabtagene ciloleucel (axi-cel, Yescarta®, Kite/Gilead) or tisagenlecleucel (Kymriah®, Novartis), starting after FDA approval from February 2018 through August 2019, as recently described by Wudhikarn et al15. All patients received CAR T-cells after lympho-depleting chemotherapy, according to the FDA recommendations for LBCL, and protocol directed for B-ALL, as previously described.12 Demographic, clinical, and laboratory data were retrospectively extracted from electronic medical records. CRS and ICANS were graded according to the ASTCT grading system,16 and were considered severe if grade ≥3. CRS and ICANS data were collected prospectively for patients with LBCL. Since the B-ALL cohort was treated before the introduction of the ASTCT grading, a chart review was conducted to retrospectively grade CRS and ICANS, as previously described.17 For patients with lymphoma, disease burden was evaluated based on the last imaging before CAR T-cells infusion. This was preformed after treatment in 43 of 44 patients who received bridging therapy. One patient with progressive stage 4 disease received a brief course of steroids and was considered as having high disease burden in the absence of re-imaging. To calculate EASIX/s-EASIX/m-EASIX formulas, we collected all available LDH, CRP, creatinine and platelets values starting from day -14 to day +14 after CAR T-cells infusion, for each patient. Written informed consent for treatment was obtained from all patients. Approval for this retrospective review was obtained from Memorial Sloan Kettering Cancer Center’s Institutional Review and Privacy Board. The study was conducted in accordance with the Declaration of Helsinki.

Aims of the Study

The study’s primary objective was to investigate if the EASIX/s-EASIX/m-EASIX formulas would predict severe CRS or ICANS. The secondary objectives included: 1) exploration of the correlation of EASIX/s-EASIX/m-EASIX scores with the rates of complete response (CR) and best overall response (ORR) to CAR T-cells therapy; 2) selection of the formula with the best predictive power for the primary outcome; 3) assessment of the selected formula to predict severe CRS and ICANS in disease subgroups (B-ALL and LBCL).

Statistical Analysis

As published in the original report, a log transformation using base 2 (log2) was applied to all the EASIX/s-EASIX/m-EASIX scores to reduce skewness. A one-unit increase in log2 EASIX was associated with a doubling (one-fold increase) of EASIX on the original scale. EASIX/s-EASIX/m-EASIX scores were calculated at specific timepoints considered clinically relevant for estimating and managing the risk of toxicities: pre-infusion, at start of lymphodepletion, and day
-1 (or day 0, if not available); and post-infusion, at day +1, +3, and at onset of CRS symptoms. Best overall response was assessed between day +30 and day +90 after infusion. For patients with LBCL, response was assessed by PET CT or biopsy, based on clinical indication, and reported according to the Lugano Criteria. Patients with B-ALL who achieved morphologic CR with persistence of minimal residual disease (MRD), as assessed by multiparameter flow cytometry, were included in the CR group. The threshold for MRD negativity was defined as < 0.01% of bone marrow blasts.

Univariate logistic regression was performed to investigate the association between predictors of interest and the outcomes of severe CRS and ICANS. Multivariate logistic regression was performed based on univariate significance and clinical judgment: disease subtype (B-ALL vs LBCL), age as a continuous variable, and burden of disease (high vs. low). Logistic regression was also utilized for the endpoint of CR, to evaluate which EASIX formula was associated with response. Receiver operating characteristic (ROC) curves were built and areas under the curve (AUC) were evaluated to select the formula with the highest discriminatory ability. Comparison of AUCs at specific timepoints was performed with ROC tests. All analyses and graphics were produced using R version 3.6.2.

Results

Patients characteristics and CRS-ICANS rates

One hundred eighteen patients were included: 53 with B-ALL, who received 1928z CAR T-cells (NCT01044069) and 65 with LBCL, 44 (68%) treated with axi-cel, and 21 (32%) with tisagenlecleucel, based on physician’s preference. Demographic and clinical characteristics of the patients at time of CAR T-cells infusion are outlined in table 1. Median age was 58 (range 20-86), 44 (range 22-74) and 64 years (range 20-86) in all patients, patients with B-ALL and with LBCL, respectively. The pre-infusion ECOG score was 0-1 in 93% of patients in the overall cohort and in 100% and 86% of patients with B-ALL and lymphoma, respectively. More than half of the population (66%) had a high disease burden before CAR T-cells infusion, defined as bone marrow (BM) blasts percentage >5% for B-ALL (62% of cases) and stage 3-4 and/or bulky disease for patients with LBCL (69% of cases).

Supplemental Table 1 summarizes the rates of CRS and ICANS by ASTCT grading, stratified by disease subtype and CAR T-cell product received. Eighty-three percent of patients had CRS of
any grade. Grade ≥3 CRS occurred in 19% of patients. Forty-seven percent of the overall population developed ICANS of any grade, with 36% of patients developing grade ≥3 ICANS. Median onset of CRS and ICANS symptoms was 2 days (IQR 1-4, range 0-12) and 6 days (IQR 4-8, range 1-21), respectively. Median onset of severe CRS symptoms was 4 days (IQR 2-7, range 0-12). Data to calculate time of median onset of severe symptoms of ICANS was available only in a minority of patients and was thus not reported.

**EASIX/s-EASIX/m-EASIX are associated with onset and severity of CRS and ICANS**

EASIX, s-EASIX, and m-EASIX were calculated for each patient at a) pre-infusion timepoints (start of lymphodepletion and day -1); b) early post-infusion timepoints (day +1 and +3); and c) at day of onset of CRS. As primary outcome, we investigated the association between the different EASIX scores calculated at various timepoints and the onset of CRS and ICANS, and of severe CRS and ICANS. Median scores at different timepoints stratified by occurrence or non-occurrence of CRS are shown in supplemental Table 2.

We found that all three formulas, when calculated pre-infusion (start of lymphodepletion and day -1) were associated with the occurrence of any grade of CRS (supplemental Table 3) and, more importantly, with severe CRS (Figure 1; e.g. at day -1, per log2 increase: EASIX OR 1.51, 95%CI 1.12-2.09, p. 0.008; s-EASIX OR 1.6, 95%CI 1.19-2.23, p. 0.003; m-EASIX OR 1.26, 95%CI 1.07-1.50, p. 0.009). Conversely, EASIX/s-EASIX/m-EASIX scores at pre-infusion timepoints were not associated with the onset of ICANS (supplemental Table 3). When we looked at early timepoints post-infusion (day +1 and +3), a significant association was found between all three EASIX scores and CRS of any grade and ICANS of any grade (supplemental Table 3). Moreover, all 3 formulas were associated with the onset of both severe CRS (e.g. at day +1, per log2 increase: EASIX OR 1.56, 95%CI 1.15-2.18, p. 0.006; s-EASIX OR 1.65, 95%CI 1.22-2.34, p. 0.002; m-EASIX OR 1.31, 95%CI 1.10-1.60, p. 0.004) and severe ICANS (e.g. at day +3, per log2 increase: EASIX OR 1.5, 95%CI 1.17-1.96, p. 0.002; s-EASIX OR 1.55, 95%CI 1.20-2.04, p. 0.001; m-EASIX OR 1.36, 95%CI 1.15-1.65, p. <0.001) (Figure 1).

**Lower platelet counts and higher CRP are associated with onset of severe CRS and ICANS**

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To investigate the contribution of the individual variables included in the formulas, we explored the association of creatinine, LDH, platelets, and CRP with the onset of severe CRS and ICANS (Table 2). Increased CRP levels at all timepoints were associated with the onset of severe CRS (e.g., per log increase, at start of lymphodepletion OR 1.87, 95%CI 1.21-3.18, p. 0.01) and severe ICANS (e.g., per log increase, at day +3 OR 2.19, 95%CI 1.44-3.63, p. <0.001). Higher platelet counts were associated with reduced toxicity: at all timepoints higher platelets values were associated with a lower odds of severe CRS (e.g., per 10 units increase, at start of lymphodepletion OR 0.87, 95%CI 0.80-0.94, p.<0.001), and of severe ICANS (e.g., per 10 units increase, at day +3 OR 0.85, 95%CI 0.78-0.92, p. <0.001). In contrast, no association was found between LDH or creatinine levels and severe CRS and ICANS.

**ROC curve analysis and selection of the m-EASIX formula as the best predictor of CAR T-cells outcomes**

To evaluate the model's performance, a ROC curve analysis was conducted with an estimation of the AUCs for EASIX/s-EASIX/m-EASIX scores calculated at all the timepoints that showed a significant association with severe CRS or ICANS in the logistic regression analysis (Figure 2). The EASIX/s-EASIX/m-EASIX scores were confirmed to be good predictors for severe CRS at start of lymphodepletion (AUCs 77.3% / 82.1% / 80.4%), at day -1 (AUCs 71.8% / 74.5% / 73%), day +1 (AUCs 72.4% / 76.4% / 75.4%), and day +3 (AUCs 79.7% / 80.9% / 80.2%) (Figure 2, a-b-c-d). m-EASIX values at day +3 showed the highest discriminatory ability for severe ICANS (AUCs 68% / 68.4% / 73% for EASIX/s-EASIX/m-EASIX, respectively) (Figure 2, f). No clear superiority emerged of one formula over the others in comparing the AUCs at all timepoints (Figure 2a-f).

Since the ROC curve analysis did not identify a superior formula – considering that platelets and CRP levels were shown to have the most relevant individual role in the prediction of both severe CRS and severe ICANS in the single variable analysis, and that m-EASIX on day +3 had the strongest association with severe ICANS compared to the other formulas - we selected the m-EASIX score (which includes CRP) as the most comprehensive formula for the early prediction of severe CRS and ICANS in patients treated with CAR T-cells.

**m-EASIX as an early biomarker of severe CRS and ICANS**
For the remainder of the analysis, we focused on the m-EASIX formula. Figure 3 displays the distribution of m-EASIX values at different timepoints across all patients, showing a progressive increase in median values for patients presenting with no symptoms, mild (CRS or ICANS grade 1-2) or severe (CRS or ICANS grade 3-5) symptoms of CRS and ICANS.

To better characterize the selected formula, we explored the association between m-EASIX and baseline patients’ characteristics. At all timepoints, lower m-EASIX levels were correlated with low disease burden (e.g. at start of lymphodepletion: beta-coefficient -3.4, 95%CI -4.9,-1.9, p.<0.001), while occurrence of fever during the 14 days preceding CAR T-cells infusion was associated with higher m-EASIX levels (e.g., at day -1: beta-coefficient 3.5, 95%CI 2.1, 5.0, p. <0.001). No consistent association was found with age, diagnosis of B-ALL vs. LBCL, and ECOG 0-1 vs. ≥2 (supplemental Table 4). In a multivariate analysis for prediction of severe CRS, after adjusting for other pre-infusion risk factors for severe CRS (disease type, disease burden, age) m-EASIX calculated at pre-infusion timepoints remained a significant predictor of severe CRS (at day of lymphodepletion: OR 1.38, 95%CI 1.11-1.85, p. 0.01; at day -1: OR 1.21, 95%CI 1.00-1.48, p. 0.05). m-EASIX achieved the highest AUC for the association with severe CRS at the start of lymphodepletion. At that timepoint, an m-EASIX cutpoint of 6.2, where specificity was 76% and sensitivity 80%, showed a negative predictive value of 96.43% for prediction of CRS grade 3 or above.

We further compared m-EASIX with clinical variables, including CRP and fever. m-EASIX on day -1 pre-infusion was superior to CRP alone in predicting severe CRS (ROC comparison for m-EASIX vs. CRP 0.025). No difference with CRP, however, was seen at the start of lymphodepletion (ROC comparison 0.059). When comparing with fever, m-EASIX on days +1 and +3 post-infusion was not superior to fever in predicting severe CRS or ICANS (ROC comparison for severe CRS: m-EASIX vs. Fever days +1 and +3: 0.284 and 0.367, respectively. ROC comparison for severe ICANS: m-EASIX vs. Fever days +1 and +3: 0.237 and 0.492, respectively). However, in 42 patients who developed fever on day +1, only 12 developed severe CRS and 23 severe ICANS. Therefore, day +1 fever did not necessarily translate into severe symptoms.

Subanalysis for m-EASIX and severe CRS and ICANS by disease type (B-ALL and LBCL)

Lastly, we explored the impact of m-EASIX scores on severe CRS and ICANS risk by disease type (supplemental Table 5). In the 53 patients with B-ALL, 15 patients (28%) had severe CRS
and 24 (45%) had severe ICANS. m-EASIX remained a significant predictor of severe CRS at all timepoints (e.g., per log2 increase, at start of lymphodepletion: OR 5.33, 95%CI 1.77-62.1, p. 0.043, at day -1: OR 1.45, 95%CI 1.08-2.12, p. 0.027), and of severe ICANS at day +3 (per log2 increase, at day +3: OR 1.5, 95%CI 1.10-2.21, p. 0.02). In the 65 patients with LBCL, severe events were less frequent (7 cases of severe CRS and 14 of severe ICANS). Only m-EASIX calculated at day +3 retained statistical significance for predicting severe CRS and severe ICANS (per log2 increase, OR 1.34, 95%CI 1.03-1.86, p. 0.045 and OR 1.25, 95%CI 1.03-1.58, p. 0.038).

**m-EASIX but not EASIX or s-EASIX is associated with CAR T cells efficacy**

All patients were evaluable for disease response assessment, except for 1 patient who died with grade 5 CRS on day +5. Out of 117 patients, 89 patients (76%) responded to treatment between day 30 and 90, with 79 (67%) achieving a CR as the best response. Among patients with B-ALL, 44 (83%) achieved a CR, which was MRD negative in 35 patients (66%). Thirty-five patients (54%) with NHL achieved a CR by day 90 after infusion.

When we explored the association of the three scores with achieving a CR as best overall response, we found that higher m-EASIX levels were associated with a reduced odds of response to CAR T-cells (per log2 increase, at day -1: OR 0.81, 95%CI 0.69-0.93, p. 0.004; at day +1: OR 0.82, 95%CI 0.70-0.94, p. 0.007; at day +3: OR 0.84, 95%CI 0.72-0.98, p. 0.031; at the first day of CRS: OR 0.67, 95%CI 0.52-0.84, p. 0.001), suggesting that patients with higher m-EASIX scores were less likely to achieve a CR (Table 3). Based on the AUCs, m-EASIX scores in our population were shown to have a moderate discriminatory power for achieving a CR (AUCs 62.2%, 68.1%, 66.8%, 66.8%, at start of lymphodepletion, day -1, +1, and +3, respectively). No clear association with disease response was found for EASIX and s-EASIX scores.

**Discussion**

In this study, we explored the role of the EASIX formula [LDH x Creatinine / PLT], a biomarker for endothelial damage in allo-HCT recipients, in predicting severe CRS and ICANS in patients with LBCL and B-ALL treated with CAR T-cells. In addition to EASIX, we tested two new revised formulas, the simplified-EASIX (s-EASIX), which excludes creatinine, and the modified-EASIX...
(m-EASIX), which replaces creatinine with CRP (mg/dL). We found that all three formulas were associated with severe CRS prior to CAR T-cells infusion (at day of lymphodepletion and day -1) and early post-infusion (day +1 and day +3), preceding the onset of severe symptoms. Early post-infusion, the three formulas also had a moderate discriminatory ability for severe ICANS. When we assessed the individual role of each variable included in the formulas, low platelets and high CRP levels at all pre- and post-infusion timepoints emerged as the variables most correlated with the development of severe CRS and ICANS.

Thrombocytopenia is a result of endothelial damage and complement activation in many diseases. In patients treated with CAR T-cells, low platelets can be multifactorial, related to higher disease burden, heavy pre-treatment including autologous or allogeneic transplant, and systemic inflammatory syndrome. In patients with both B-ALL and aggressive lymphomas treated with various CAR T-cells products, a lower platelet nadir has been shown to be independently correlated with higher grades of CRS. In other reports, platelet counts at time of CAR T-cells infusion were shown to be significantly lower in patients who then developed severe neurotoxicity compared to those who had mild symptoms. At CAR T-cells peak expansion, endothelial activation represented by high Ang-2 levels and Ang-2/Ang-1 ratio has been associated with severe CRS. Similarly, high Ang-2 levels and Ang-2/Ang-1 ratio have been reported one week after CAR T-cells infusion, but also before the start of lymphodepletion, in patients who subsequently developed high-grade neurotoxicity, suggesting that a pro-endothelial damage state can precede CAR T-cells infusion. As platelets are one of the few sources of the endothelial stabilizing cytokine Ang-1, patients with severe thrombocytopenia before and early after CAR T-cells infusion might be more prone to CRS and ICANS-related endothelial activation and damage.

High CRP levels have previously been reported in patients with severe CRS and ICANS. Early elevation of CRP after CAR T-cells infusion was associated with grade 4–5 CRS in patients with B-ALL and other lymphoid neoplasms; however, its utility as an early biomarker of severe CRS has not been proven. While in one report, peak CRP levels were shown to be higher in patients who experienced high-grade neurotoxicity, in another, CRP levels peaked in all patients before the development of neurologic symptoms, but failed at discriminating between patients with mild vs. severe symptoms. Conversely, unlike in Karschnia et al. who reported elevated (≥400 U/L) LDH levels at baseline in 69% of patients with severe neurotoxicity, we did not find a correlation between LDH levels
and severe CRS or ICANS. Endothelial activation leads to release of LDH from endothelial cells as well as to a higher turnover of circulating cells, such as platelets and leukocytes, resulting in elevated LDH serum levels. LDH is also a marker for neoplastic cells proliferation and high disease burden, which have been implicated in the development of severe CRS and ICANS. High LDH has also been correlated with decreased survival after CAR T cells infusion. The absence of an association between LDH and CAR T-cells related toxicities in our sample might be a result of the small sizes of the two populations involved.

Based on our findings, we chose the m-EASIX as the most clinically relevant formula for the prediction of severe toxicities. At present several laboratory parameters, cytokines, and clinical characteristics have been associated with the development of CRS and ICANS. However, no specific biomarker has been identified for use in clinical practice as an effective predictor. The use of currently known biomarkers has been limited by the fact that the majority of studies involve non-routinely available cytokines. A clear strength of the m-EASIX is that it includes laboratory parameters that are ubiquitously available and often routinely assessed in clinical practice for patients treated with CAR T-cells. Besides, the formula can be easily calculated at the bedside. Moreover, the m-EASIX proved to effectively correlate with severe CRS and ICANS at clinically actionable timepoints, before and early after CAR T-cells infusion. Increasing m-EASIX scores calculated pre-infusion (at lymphodepletion and day -1) were predictive of CRS grade 3 or above, with an m-EASIX cutpoint of 6.2 at start of lymphodepletion showing a 96.43% negative predictive value for severe CRS. In the clinical setting, patients with m-EASIX values below 6.2 at the start of lymphodepletion might be considered for outpatient treatment as their risk of developing severe symptoms is predicted to be low. Conversely, a higher pre-infusion m-EASIX score might help in selecting patients requiring inpatient admission on the day of infusion for careful monitoring, or patients who might be candidates for clinical trials of prophylactic or preemptive treatment of toxicities. In our population, CRS median onset was day +2, with severe symptoms occurring at a median of day +4, in line with previous reports. Early after infusion (day +1 and +3), higher m-EASIX values were associated with both severe CRS and severe ICANS, and this could be used for similar interventions. The fact that only post-infusion m-EASIX levels are associated with severe ICANS might be explained because most patients develop neurologic symptoms only after having experienced some degree of CRS. However, it is not clear if higher grades of CRS are more predictive of ICANS, thus the m-EASIX might help in identifying those patients at higher risk.
The selection of the m-EASIX as the best score was also guided by the fact that it was the only formula that included CRP and was also associated with complete response. Recently, CRP levels on day 0 have been shown to correlate with response to axicabtagen ciloleucel. Interestingly, higher m-EASIX levels were associated with severe CRS and ICANS, and conversely with lower chances of achieving a complete response. This highlights the potential role of CRP as a marker of disease burden besides systemic inflammation. Higher disease burden and presence of fever before CAR T-cells infusion were in fact associated with higher m-EASIX levels at all timepoints. In general, disease burden is known to be a risk factor for CRS and patients with higher disease burden are also at higher risk of not responding. Furthermore, patients who develop mild or no CRS have been shown to effectively respond to CAR T-cells. Therefore, experiencing CRS might not necessarily be a requirement for a good response to CAR T-cells. The association between m-EASIX and response suggests that the combination of disease/patient/pre-treatment factors, such as LDH and platelets, and of markers of inflammation due to disease, such as CRP, provides better prediction of ultimate disease response, rather than the factors in isolation.

Our study has some limitations. The sample size is in line with recent single-center retrospective reports on patients treated with CAR T-cells. However, it is limited by combining patients affected by two different diseases and treated with different products, which might represent confounding factors. As such, while the results from the whole population were confirmed in the B-ALL subgroup analysis, we were not able to prove the same for the LBCL patients, likely related to the paucity of CRS/ICANS events in this subgroup. Finally, further validation in external cohorts is warranted. To conclude, we propose that the m-EASIX score should be further investigated as an early biomarker of severe CRS and ICANS in larger datasets. The m-EASIX could provide a clinical tool for discriminating patients at higher risk for severe toxicities at clinically actionable timepoints (before or early after infusion) to guide differential individualized management.

Data sharing statement

Deidentified individual participant data will be available by specific email request to the corresponding author: peralesm@mskcc.org.

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Authorship and Conflicts of Interest

MP, MSE, LYSS and MAP designed the study and wrote the manuscript. MP and MSE collected the data and conducted the analysis. MLS, AAT, TJ, JR and MM participated in data collection. JF and SMD conducted the statistical analysis. RS participated into the data analysis. CB, RB, PBD, CD, EH, EM, BS, SG, MLP, CSS, MS, GS and JHP took care of the patients. All the authors reviewed and approved the manuscript.

BS has consulted for Juno Therapeutics, Celgene/BMS, and Novartis and has served on advisory boards for Kite/Gilead and Janssen Pharmaceuticals. CB has consulted for and served on advisory boards for Juno Therapeutics. RB has consulted for Celgene and has consulted for, has patents & royalties and received research funding from Juno Therapeutics. PBD has served on advisory boards for Kite – A Gilead Company. SG has consulted for and received research funding from Amgen, Actinium, Celgene, Johnson & Johnson, Takeda; has consulted for Jazz Pharmaceuticals, Novartis, Kite, Spectrum Pharmaceuticals; has received research funding from Miltenyi. TJ has consulted for Targeted Oncology and served on advisory board for Bristol Myers Squibb and CareDx. MLP has consulted for Noble Insights and Merck & Co Inc; has served on advisory boards for STRAXIMM, Kite Pharmaceuticals, Pharmacyclics and Seres Therapeutics; has served on the Speakers Bureau for Hemedicus; has equity ownership for Seres Therapeutics and Evelo; has patents & royalties for MSKCC (IP for Juno and Seres Therapeutics). CSS has served as a paid consultant on advisory boards for: Juno Therapeutics, Sanofi-Genzyme, Spectrum Pharmaceuticals, Novartis, Genmab, Precision Biosciences, Kite/a Gilead Company, Celgene/BMS, Gamida Cell, Karyopharm Therapeutics and GSK. He has received research funds for clinical trials from: Juno Therapeutics, Celgene/BMS, Bristol-Myers Squibb, Precision Biosciences and Sanofi-Genzyme. MS reports research support/funding: Angiocrine Bioscience, Inc. Consultancy: Angiocrine Bioscience, Inc.; Omeros Corporation; McKinsey & Company. Ad-hoc advisory board: Kite – A Gilead Company; One-time
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### Table 1. Patients characteristics

| Patients                          | Overall (118) | B-ALL (53) | LBCL (65) |
|----------------------------------|---------------|------------|-----------|
| **Age years – median (range)**   | 58 (20-86)    | 44 (22-74) | 64 (20-86) |
| **Sex male – n (%)**             | 84 (71)       | 39 (74)    | 45 (69)   |
| **ECOG pre-infusion – n (%)**    |               |            |           |
| 0-1                              | 109 (93)      | 53 (100)   | 56 (86)   |
| 2-4                              | 7 (6)         | 0          | 7 (11)    |
| Not available                    | 2 (1)         | 0          | 2 (3)     |
| **Disease burden pre-infusion – n (%)** | | | |
| High                             | 78 (66)       | 33 (62) *  | 45 (69) **|
| Low                              | 37 (31)       | 20 (38) ^  | 17 (26) ^^|
| N/A °                            | 3 (3)         | 0          | 3 (5)     |
| **IPI pre-infusion – n (%)**     |               |            |           |
| Low/Intermediate Low (0-2)       | -             | -          | 39 (60)   |
| High/Intermediate High (3-5)     | -             | -          | 22 (35)   |
| Not available                    | -             | -          | 3 (5)     |
| **Fever pre/during lymphodepletion – n (%)** | | | |
| 23 (19)                          | 10 (19)       | 13 (20)    |
| **Lymphodepleting chemotherapy – n (%)** | | | |
| Fludarabine/Cyclophosphamide     | 72 (61)       | 10 (19)    | 62 (95)   |
| High-dose cyclophosphamide       | 43 (36)       | 43 (81)    | -         |
| Bendamustine                     | 3 (3)         | -          | 3 (5)     |
| **CAR T cell product – n (%)**   |               |            |           |
| CD1928z                          | 53 (45)       | 53 (100)   | 0 (0)     |
| Axicabtagene Ciloleucel          | 44 (37)       | 0 (0)      | 44 (68)   |
| Tisagenlecleucel                 | 21 (18)       | 0 (0)      | 21 (32)   |

*B-ALL, B-cell Acute Lymphoblastic Leukemia; LBCL, Large Cell B-cell Lymphoma; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; N/A, not applicable; BM, bone marrow; CR, complete remission.

*BM Blasts >5%; ** Stage 3-4 and/or Bulky >6cm; ^ BM Blasts <5%; ^^Stage 1-2; °patients in CR at infusion
Table 2. Association of individual variables included in EASIX scores and severe CRS/ICANS

| Severe CRS          | At start of lymphodepletion | Variable | Median (range) | N   | OR  | 95% CI        | p-value |
|---------------------|-----------------------------|----------|----------------|-----|-----|---------------|---------|
| LDH* (U/L)          |                             | 227 (110, 8255) | 112 | 1.17 | 0.55, 2.24 | 0.7     |
| Creatinine (mg/dL)  |                             | 0.80 (0.30, 2.00) | 118 | 0.05 | 0.00, 0.49 | 0.018   |
| PLTs° (k/ul)        |                             | 14 (1, 49) | 118 | 0.87 | 0.80, 0.94 | <0.001  |
| CRP* (mg/L)         |                             | 1.3 (0.0, 28.5) | 84  | 1.87 | 1.21, 3.18 | 0.01    |
| At day – 1          |                             | Variable | Median (range) | N   | OR  | 95% CI        | p-value |
| LDH* (U/L)          |                             | 232 (106, 4110) | 112 | 1.66 | 0.81, 3.30 | 0.15    |
| Creatinine (mg/dL)  |                             | 0.70 (0.30, 1.60) | 118 | 0.22 | 0.02, 1.68 | 0.2     |
| PLTs° (k/ul)        |                             | 12 (1, 44) | 118 | 0.87 | 0.80, 0.94 | 0.001   |
| CRP* (mg/L)         |                             | 2.0 (0.0, 28.1) | 103 | 1.64 | 1.13, 2.51 | 0.014   |
| At day + 1          |                             | Variable | Median (range) | N   | OR  | 95% CI        | p-value |
| LDH* (U/L)          |                             | 207 (95, 2645) | 112 | 1.63 | 0.75, 3.42 | 0.2     |
| Creatinine (mg/dL)  |                             | 0.70 (0.30, 1.60) | 118 | 0.29 | 0.03, 2.35 | 0.3     |
| PLTs° (k/ul)        |                             | 11 (1, 38) | 118 | 0.84 | 0.76, 0.92 | <0.001  |
| CRP* (mg/L)         |                             | 2.2 (0.1, 27.0) | 104 | 1.83 | 1.20, 2.98 | 0.009   |

| Severe ICANS        | At day + 1                   | Variable | Median (range) | N   | OR  | 95% CI        | p-value |
|---------------------|-----------------------------|----------|----------------|-----|-----|---------------|---------|
| LDH* (U/L)          |                             | 207 (95, 2645) | 112 | 1.39 | 0.71, 2.70 | 0.3     |
| Creatinine (mg/dL)  |                             | 0.70 (0.30, 1.60) | 118 | 0.3  | 0.04, 1.70 | 0.2     |
| PLTs° (k/ul)        |                             | 11 (1, 38) | 118 | 0.91 | 0.85, 0.97 | 0.004   |
| CRP* (mg/L)         |                             | 2.2 (0.1, 27.0) | 104 | 1.54 | 1.11, 2.21 | 0.013   |
| At day +3           |                             | Variable | Median (range) | N   | OR  | 95% CI        | p-value |
| LDH* (U/L)          |                             | 202 (96, 1810) | 103 | 1.17 | 0.54, 2.46 | 0.7     |
| Creatinine (mg/dL)  |                             | 0.70 (0.30, 1.80) | 118 | 0.91 | 0.21, 3.52 | 0.9     |
| PLTs° (k/ul)        |                             | 8 (1, 34) | 118 | 0.85 | 0.78, 0.92 | <0.001  |
| CRP* (mg/L)         |                             | 7 (0, 33) | 105 | 2.19 | 1.44, 3.63 | <0.001  |

OR, Odds Ratio; CI, Confidence Interval; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; LDH, Lactic Dehydrogenase; PLTs, platelets; CRP, C-reactive protein.

* log-transformation of raw LDH and CRP values was applied to calculate the ORs
° PLT values were scaled by 10 units to calculate the ORs
Table 3. Correlation of m-EASIX and achievement of CR

| Formula | N  | OR  | 95% CI      | p-value |
|---------|----|-----|-------------|---------|
|         |    |     |             |         |
| **At lymphodepletion** |    |     |             |         |
| EASIX   | 111| 0.87| 0.67, 1.12  | 0.3     |
| s-EASIX | 111| 0.87| 0.67, 1.12  | 0.3     |
| m-EASIX | 81 | 0.9 | 0.78, 1.03  | 0.13    |
| **At day -1** |    |     |             |         |
| EASIX   | 111| 0.77| 0.59, 1.00  | 0.053   |
| s-EASIX | 111| 0.79| 0.61, 1.02  | 0.078   |
| m-EASIX | 97 | 0.81| 0.69, 0.93  | 0.004   |
| **At day +1** |    |     |             |         |
| EASIX   | 111| 0.79| 0.61, 1.02  | 0.078   |
| s-EASIX | 111| 0.81| 0.62, 1.04  | 0.1     |
| m-EASIX | 97 | 0.82| 0.70, 0.94  | 0.007   |
| **At day +3** |    |     |             |         |
| EASIX   | 102| 0.84| 0.65, 1.07  | 0.2     |
| s-EASIX | 102| 0.83| 0.65, 1.06  | 0.14    |
| m-EASIX | 92 | 0.84| 0.72, 0.98  | 0.031   |
| **At day of CRS** |    |     |             |         |
| EASIX   | 88 | 0.81| 0.60, 1.08  | 0.2     |
| s-EASIX | 88 | 0.82| 0.61, 1.09  | 0.2     |
| m-EASIX | 78 | 0.67| 0.52, 0.84  | 0.001   |

OR = Odds Ratio, CI = Confidence Interval
CR = Complete Response, for B-cell Acute Lymphoblastic Leukemia including also patients with positive minimal residual disease as assessed by flow-cytometry
Figure Legends

Figure 1. Association of EASIX/s-EASIX/m-EASIX with severe CRS and severe ICANS.
This figure shows a Forest Plot for Odds Ratios (OR) and 95% Confidence Intervals (95% CI) for prediction of severe cytokine release syndrome (CRS, above) and immune effector cell-associated neurotoxicity syndrome (ICANS, below) by EASIX/s-EASIX/m-EASIX calculated at pre-infusion (lymphodepletion and day-1) and post-infusion (day +1 and +3) timepoints.

Figure 2. Prediction of severe CRS and ICANS by EASIX/s-EASIX/m-EASIX.
ROC curves are shown at all pre- and post-infusion timepoints for severe CRS (a-b, c-d) and ICANS (e-f). AUCs are calculated for all curves and compared in pairs with ROC tests at all timepoints.

Figure 3. Distribution of m-EASIX levels across CRS and ICANS subgroups.
The boxplots summarize median and interquartile range (IQR) of m-EASIX levels at day of lymphodepletion (a), day -1 (b), day +1 (c) and day +3 (d) for patients presenting with no (grey), mild (light yellow) or severe (yellow) CRS; and at day +1 (e) and day + 3 (f) for patients with no (grey), mild (light blue) or severe (blue) ICANS. CRS and ICANS were defined mild if grade 1-2 and severe if grade 3-5.
Severe CRS

| Lymphodepletion (N=112) | Day -1 (N=112) | 1.34 (1.01–1.85) |
| Day +1 (N=112) | 1.51 (1.12–2.09) |
| Day +3 (N=103) | 1.89 (1.39–2.7) |

| Lymphodepletion (N=81) | Day -1 (N=98) | 1.32 (1.08–1.68) |
| Day +1 (N=98) | 1.26 (1.07–1.5) |
| Day +3 (N=93) | 1.56 (1.24–2.07) |

| Lymphodepletion (N=112) | Day -1 (N=112) | 1.49 (1.11–2.1) |
| Day +1 (N=112) | 1.65 (1.22–2.34) |
| Day +3 (N=103) | 1.92 (1.4–2.78) |

Severe ICANS

| Lymphodepletion (N=112) | Day -1 (N=112) | 1.11 (0.86–1.44) |
| Day +1 (N=112) | 1.2 (0.93–1.56) |
| Day +3 (N=103) | 1.36 (1.05–1.89) |

| Lymphodepletion (N=81) | Day -1 (N=98) | 1.1 (0.95–1.27) |
| Day +1 (N=98) | 1.2 (0.98–1.29) |
| Day +3 (N=93) | 1.36 (1.15–1.65) |

| Lymphodepletion (N=112) | Day -1 (N=112) | 1.25 (0.97–1.65) |
| Day +1 (N=112) | 1.33 (1.03–1.75) |
| Day +3 (N=103) | 1.55 (1.2–2.04) |
Severe CRS

A
At lymphodepletion

B
At day -1

C
At day +1

D
At day +3

EASIX vs. sEASIX: 0.043
EASIX vs. mEASIX: 0.064
sEASIX vs. mEASIX: 0.144

EASIX vs. sEASIX: 0.088
EASIX vs. mEASIX: 0.816
sEASIX vs. mEASIX: 0.354

EASIX vs. sEASIX: 0.711
EASIX vs. mEASIX: 0.911
sEASIX vs. mEASIX: 0.974

Severe ICANS

E
At day +1

F
At day +3

EASIX vs. sEASIX: 0.256
EASIX vs. mEASIX: 0.110
sEASIX vs. mEASIX: 0.323

EASIX vs. sEASIX: 0.723
EASIX vs. mEASIX: 0.522
sEASIX vs. mEASIX: 0.472

- EASIX - s-EASIX - m-EASIX
