ESMO '22 Abstract #128P: Histopathology (HP) biomarkers confirm Leukocyte Interleukin Injection (LI) treatment (Tx) outcome in naïve locally advanced primary head & neck squamous cell carcinoma (SCCHN) the IT-MATTERS Study (Clinicaltrials.gov NCT01265849)

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ABSTRACT

Background: In a randomized controlled pivotal Phase 3 pre-surgery administration of investigational proinflammatory biologic (LI) with CIZ (single low dose cyclophosphamide IV, indomethacin [po tid] and Zinc multivitamins [po, daily]) + Standard of Care (SOC) to treatment (Tx) naïve resectable locally advanced oral and soft-palate SCCHN subjects, resulted in significantly prolonged overall survival (OS) in the NCCN Guidelines defined low risk (LR) for recurrence intent to treat (ITT) population vs SOC alone.

Methods: Available HP samples (453 of 923 ITT; 210 of 380 LR ITT) meeting entry criteria (AJCC Stage III/IVa OSCC, soft-palate SCCHN, Tx naïve) randomized 3:1:3 to Tx arms LI (+/- CIZ) + SOC or SOC alone. LI was injected (1/2 daily dose) 200IU peritumorally and 200IU peri-lymphatically, for 3-consecutive weeks, before surgery. All study subjects were to receive SOC (per NCCN, LR-> RTx; high risk (HR)-> CRTx, post-surgery). Follow-up was comparable for all Tx groups (56-57 months median per Tx group). Tumor HP (obtained at surgery) samples were stained/quantitated for 20 biomarkers (5 tumor cell, 15 tumor microenvironment), 2 ratios, and 14 marker combinations all prospectively defined, including low/high thresholds (positive cells/mm^2; PDL1 % positive cells) for each biomarker, ratio; combinations defined as +ve or -ve. Defined prospective interactions models (all subjects) allowed three-way interactions assessment for risk groups, biomarker/combination level and Tx, to analyze Tx efficacy for OS, PFS, LRC outcomes using proportional hazard models. Analyses were repeated for the LR group using 2-way interactions.

Results: HP samples (n=453) were representative of the overall population (n=923). For combined OS, PFS, and LRC, 21.9% LR overall and 19.4% LR group hazard ratios (HZR) significantly exceeded one-sided 2.5% chance, always favoring LI+CIZ+SOC vs SOC, whereas only 1.9% High Risk overall HZR all were within chance (i.e., could not be ruled as a significant effect).

Conclusions: Efficacy (OS, PFS, LRC) was seen for multiple biomarkers (tumor: p16, PDL1, TME: CD4, CD8, CD3, FOXP3, CD20, CD68, CD163, CD1A, immune cells: PD1, CTLA4, PDL1, and CD25), ratios (CD4/CD8, CD8/FOXP3), and pre-defined combinations confirm and support LI OS efficacy.

STUDY DESIGN:

Previously untreated locally advanced primary SCCHN patients (oral cavity including anterior tongue (only), floor of mouth, buccal mucosa (cheek), and soft palate) were consented, and consenting study subjects were enrolled following having met Inclusion/Exclusion criteria.

Patients were then randomized 3:1:3 to one of the following treatments [NOTE: LI(MK) = LI]:

- **Group 1** – LI (MK)+CIZ+SOC; n=395
- **Group 2** – LI (MK)+SOC; n=134
- **Group 3** – SOC alone (Control); n=394

Groups 1 and 3 served as the main comparator arms. Group 2 was included to assess the need for CIZ and the toxicity of LI (MK) alone (i.e., without CIZ).

Primary study objective was to assess OS superiority of LI (MK)+CIZ+SOC vs SOC alone (Control).

Secondary/Other study objectives were to assess overall survival (OS), progression-free survival (PFS), and loco-regional control (LRC) , Quality of Life, histopathological nature of cellular tumor infiltrate, and tumor response to LI (MK)+CIZ+SOC vs SOC

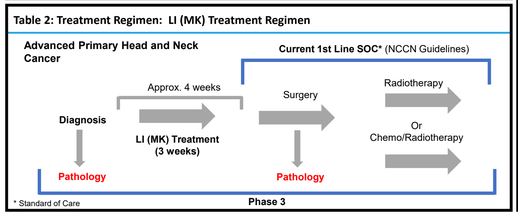
Study Power: The study had 80% power and two-sided 5% Type I error to detect a 0.721 hazard ratio which corresponded to a 10% absolute advantage at 3 years assuming exponential survival. For this comparison (Group 1 vs Group 3), the log rank test required a minimum of 298 deaths in the combined comparator arms of the study (Group 1 and Group 3). The study was designed as an event (death) driven study.

Table 1: LI (MK) Phase 3 Trial Design (Open Label – OS Primary Endpoint)

Note: The **overall survival comparison is made between Groups 1 and 3**. The primary purpose of the smaller Group 2 is to gain additional information on the mechanism of action and toxicity of LI (MK). CIZ is added to decrease tumor suppressor mechanisms and thereby is thought to increase LI (MK) effectiveness.

* CIZ: Cyclophosphamide 300 mg/m² (x1,IV, day -3); Indomethacin 25mg tid, po (day 1 to ~24 hrs (one day) prior to surgery) + 15 - 45mg Zinc (as Multivitamin) i.d., p.o.
** Surgery: complete surgical resection of primary tumor and any positive lymph nodes.
*** High risk patients are per NCCN Guidelines

Table 2: Treatment Regimen: LI (MK) Treatment Regimen



Immunohistochemistry (IHC) DAB reaction, Positive cells are brown, All IHC photos same magnification; Bar = 200 µm

Figure 1. LI(MK) Treated: High CD4 T cell density in tumor stroma

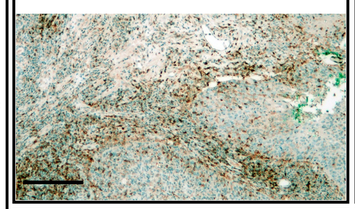


Figure 2. LI(MK) Treated: High CD8 T cell density in the tumor stroma

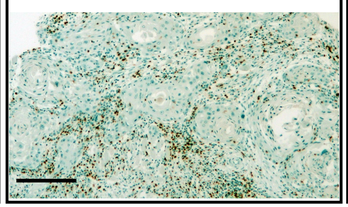


Figure 3. LI(MK) Treated: High CD68 macrophage cell density in the tumor stroma

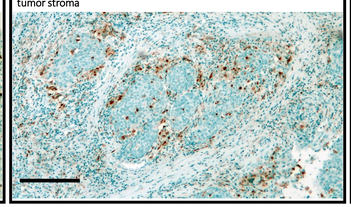


Figure 4. LI(MK) Treated: Low CD1a dendritic cell density in the tumor stroma

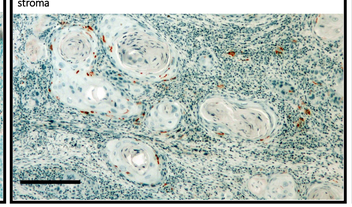


Figure 5. LI(MK) Treated: Low tumor cell PDL1 expression/negativity.

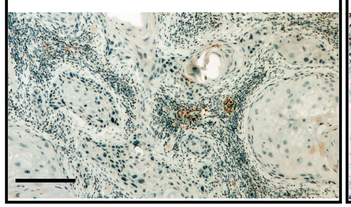


Figure 6. LI(MK) Treated: High CD20 B cell density in the tumor stroma

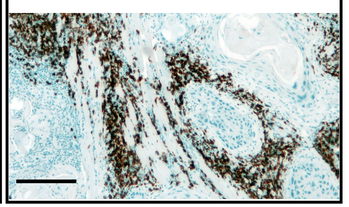


Figure 1a. SOC only (control): Low CD4 T cell density in tumor stroma

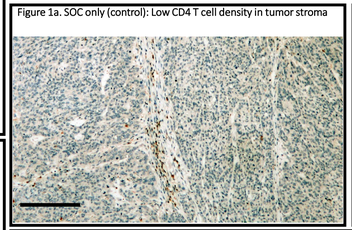


Figure 2a. SOC only (control): Low CD8 T cell density in the tumor stroma

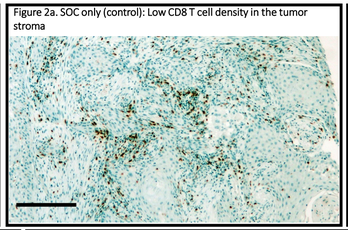


Figure 3a. SOC only (control): Low CD68 macrophage cell density in the tumor stroma

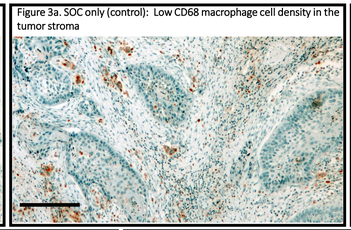


Figure 4a. SOC only (control): High CD1a dendritic cell density in the tumor stroma

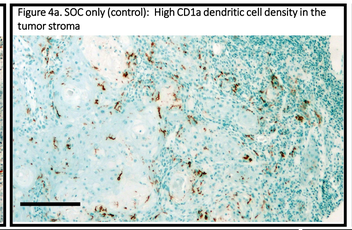


Figure 5a. SOC only (control): High tumor cell PDL1 expression

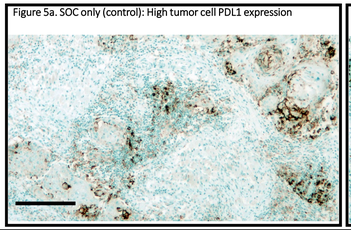
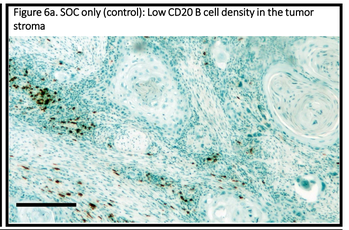


Figure 6a. SOC only (control): Low CD20 B cell density in the tumor stroma



Prospectively Defined Biomarkers (2 [L/H] or 3 levels [L/M/H])

1. p16: 10% positivity threshold
2. HLA: L<45, H≥90
3. B2M: L<40, H≥80
4. MR1: L<50, H≥100
5. TPDL1: L<1, H≥50
6. CD4: L<600, H≥1200
7. CD8: L<400, H≥800
8. CD3: L<1000, H≥2000
9. FOXP3: L<250, H≥500
10. CD20: L<250, H≥500
11. CD68: L<50, H≥100
12. CD163: L<60, H≥120
13. CD1A: L<15, H≥30
14. CD208: L<2, H≥8
15. MPOX: L<30, H≥60
16. PD1: L<10, H>20
17. CTLA4: L<9, H≥18
18. PDL1: L<0.2, H≥5
19. CD25: L<40, H>80
20. NK p46: L<2, H≥8

Prospectively Defined Ratios and Combinations

Two ratios were constructed with L, M, and H thresholds (based on above definitions of H & L, M was neither H nor L) as follows:

1. CD8/FOXP3 ratio: 1 and 2
2. CD4/CD8 ratio: 1 and 2

Fourteen combinations were constructed as follows where Y (yes) means that no marker components were low (all were either M or H = HMCOMB), while N (no) means that at least one marker component was low (L):

1. CD3+ and CD25+ All Positive
2. CD3+, CD8+, and CD25+ All Positive
3. CD3+, CD4+, and CD25+ All Positive
4. CD3+, CD4+, CD8+, and CD25+ All Positive
5. CD1A+ and TMR1+ All Positive
6. CD1A+ and NK p46+ All Positive
7. CD1A+ and CD163+ All Positive
8. CD3+, CD4+, CD25+, and NK p46+ All Positive
9. CD3+, CD4+, CD25+, and CD163+ All Positive
10. CD3+, CD4+, CD25+, CD1A+, and TMR1+ All Positive
11. CD3+, CD4+, CD25+, CD1A+, TMR1+, and CD163+ All Positive
12. CD3+, CD4+, CD25+, CD1A+, TMR1+, and NK p46+ All Positive
13. CD3+, CD4+, CD25+, CD1A+, TMR1+, CD163+, NK p46+ All Positive
14. CD3+, CD4+, CD25+, CD1A+, CD163+, and NK p46+ All Positive

Histopathology Sample Collection and Analysis

Histopathology sample reading and analysis was done in a blinded manner by Central Pathology Laboratory (CPL) and was distinct from that of each study site pathology laboratory. Since the study was conducted in multiple sites worldwide, it was not reasonable nor practical to have a central pathology laboratory to assess pathology samples and to be directly involved in study subjects' care. Thus, site qualified pathology was part of the study team involved in assessment and care of the study subjects, and, independent of the sites, the CPL performed the blinded assessment of the immunohistopathological effect of the investigational treatment on the tumor and tumor microenvironment in study subjects.

Sample Processing: Immunohistochemical markers were determined by appropriate industrial standard diagnostic primary antibodies developed by Ventana Optiview kit used on Bechmark automatic Stainer. In each case appropriate positive controls were used, most frequently lymph node slides, but in case of p16, a diagnostic p16 positive human oral squamous carcinoma sample was used. In each case, at least, 5-6 microscopic fields of each tumor sample were evaluated.

Biomarkers. P16 immunohistochemistry evaluation was based on tumor cell positivity/negativity: positivity is defined if >10% of **tumor cells** show nuclear labeling.

Tumor cell HLAI, B2M, MCR1, and PDL1 expressions were determined as % of positive tumor cells.

Tumor microenvironment (TME) markers (CD20: pan-B cell marker, CD3: pan-T cell marker, CD4: T helper cell marker, CD8: cytotoxic T cell marker, FOXP3: Treg cell marker, NKp46/NCR1/NK cells, CD68: macrophages (mostly M1), CD163: M2 macrophages, MPOX: neutrophyl granulocyte marker): evaluation was based on determination of the density of marker positive stromal immune cells expressed in **mean number/mm^2**.

CD25, PD1, PDL1, CTLA4 immune cell activation markers were assessed as **mean % of positive immune cells.** Each marker was evaluated in a minimum of 5 different peritumoral areas.

Markers expression levels were then categorized as low (L), high (H), and not low or high (medium [M]) as follows: p16: positivity threshold already as 10%, HLA: 45 and 90, B2M: 40 and 80, MR1: 50 and 100, TumorPDL1: 1 and 50, CD4: 600 and 1200, CD8: 400 and 800, CD3: 1000 and 2000, FOXP3: 250 and 500, CD20: 250 and 500, CD68: 50 and 100, CD163: 60 and 120, CD1A: 15 and 30, CD208: 2 and 8, MPOX: 30 and 60, PD1: 10 and 20, CTLA4: 9 and 18, TEMPDL1: 0.2 and 5, CD25: 40 and 80, NK p46: 2 and 8. Then ratios were constructed with low, medium, and high thresholds for prospective analyses as follows: CD8/FOXP3 ratio: 1 and 2, CD4/CD8 ratio: 1 and 2.

Statistical Methodology:

Efficacy was assessed for overall survival (OS), progression-free survival (PFS), and loco-regional control (LRC) measured from the time of study entry.

A total of 20 biomarkers, two ratios, and 14 marker combinations were prospectively defined, including low and high thresholds for each biomarker and ratio; the combinations were defined as positive or negative. Data were analyzed using proportional hazard models to assess all samples (n=453) as well as the LR group (n=210). The overall analysis simultaneously evaluated the risk group, markers, and treatment with 3-way interactions, while the LR analyses simultaneously evaluated the markers and treatment with 2-way interactions. Separately for overall and for LR, the percent with a significant one-sided p<0.025 favoring LI(MK) (corresponding to a hazard ratio <1) were compared vs 2.5% expectation as a measure of treatment efficacy.

Significant Outcomes All Favoring LI (MK) + CIZ + SOC vs SOC

	Proportion Statistically Significant, 1-sided p<0.025		
	Overall (Low Risk [LR] population)	Only LR Group (n=210)	Overall (High Risk)
Overall Survival	26/93	21/93	1/93
Progression Free Survival	17/93	16/93	2/93
Local Regional Control	18/93	17/93	2/93
Totals	61/279 (21.9%>>2.5%)	54/279 (19.4%>>2.5%)	5/279 (1.9% < 2.5%)
All advantages favored LI+CIZ+SOC vs SOC for Overall and Low Risk			

EFFICACY ENDPOINT SUMMARY:

1. ***Overall Survival: 26 significant favorable overall [>>than 2.5% by chance alone]***
   - Markers: CD4, CD8, CD3, FOXP3, CD20, CD68, CD163, CD1A, PD1, CTLA4, PDL1, CD25
   - Ratios: CD4/CD8, CD8/FOXP3
   - Combinations: CD3+ and CD25+ All Positive, HMCOMB2 - CD3+, CD8+, and CD25+ All Positive, HMCOMB3 - CD3+, CD4+, and CD25+ All Positive, HMCOMB4 - CD3+, CD4+, CD8+, and CD25+ All Positive, HMCOMB5 - CD1A+ and TMR1+ All Positive, HMCOMB6 - CD1A+ and NK p46+ All Positive, HMCOMB9 - CD3+, CD4+, CD25+, and CD163+ All Positive, HMCOMB10 - CD3+, CD4+, CD25+, CD1A+, and TMR1+ All Positive, HMCOMB14 - CD3+, CD4+, CD25+, CD1A+, CD163+, and NK p46+ All Positive

   Only one overall unfavorable disadvantage for LI(MK) + CIZ + SOC vs. SOC for High Risk and Low CD20 where HR=1.70 (two-sided 95% CI: 1.08 - 2.66) [1/93 = 1.1% <2.5% by chance alone]
2. ***Progression-free Survival: 17 significant favorable overall [>>2.5% by chance alone]***
   - Markers: CD4, CD8, CD3, FOXP3, CD20, CD68, CD163, CD1A, PD1, CTLA4, PDL1, CD25
   - Ratios: CD8/FOXP3
   - Combinations: HMCOMB1 - CD3+ and CD25+ All Positive, HMCOMB2 - CD3+, CD8+, and CD25+ All Positive, HMCOMB4 - CD3+, CD4+, CD8+, and CD25+ All Positive, HMCOMB9 - CD3+, CD4+, CD25+, and CD163+ All Positive

   Only two overall unfavorable disadvantages for LI (MK) + CIZ + SOC vs SOC for:
   * High Risk and Low CD20 where HR=1.64 (95% CI: 1.07, 2.53) [1/93 = 1.1% <2.5% by chance alone]
   * High Risk and Low CD163 where HR=1.89 (95% CI: 1.12 - 3.18) [1/93 = 1.1% <2.5% by chance alone]
3. ***Loco-regional Control: 18 significant favorable overall [>>2.5% by chance alone]***
   - Markers: CD4, CD8, CD3, FOXP3, CD20, CD1A, CD208, CTLA4, CD25
   - Ratios: CD8/FOXP3
   - Combinations: HMCOMB1 - CD3+ and CD25+ All Positive, HMCOMB2 - CD3+, CD8+, and CD25+ All Positive, HMCOMB3 - CD3+, CD4+, and CD25+ All Positive, HMCOMB4 - CD3+, CD4+, CD8+, and CD25+ All Positive, HMCOMB5 - CD1A+ and TMR1+ All Positive, HMCOMB6 - CD3+, CD4+, CD25+, and NK p46+ All Positive

   Only two overall unfavorable disadvantages for LI (MK) + CIZ + SOC vs SOC for:
   High Risk and Low CD20 where HR=1.93 (95% CI: 1.02, 3.65) [1/93 = 1.1% <2.5% by chance alone]
   High Risk and Low CD20 where HR=1.96 (95% CI: 1.01, 3.78) [1/93 = 1.1% <2.5% by chance alone]

CONCLUSIONS

- Pre-defined markers, ratios, and combinations contribute to LI(MK) efficacy for all three efficacy endpoints (OS, PFS, LRC).
- Broad representation of markers, ratios, and combinations overall and for Lower Risk (LR) for of the OS, PFS, LRC efficacy study endpoints
- There were 61 (21.9%) favorable overall and 54 (19.4%) favorable Low Risk treatment group outcomes (much beyond 2.5% chance) and only a total of five instances (1.9%) [all High Risk] having unfavorable treatment group outcome (within the realm of chance)
- **The results support the Low-Risk treatment advantage (0.68 HR, Wald p<0.05) significantly favoring LI(MK)+CIZ+ SOC vs SOC alone**

Presenter Conflict of interest/ funding statement: Dr. J. Timar, Professor - Semmelweis University – PI, on Institutional contract for the study (sponsor CEL-SCI Corp)

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