Prognostic utility of immune markers and validation of Immunoscore® in stage III colon carcinoma (CC) patients (pts) treated with adjuvant FOLFOX in a phase III trial [NCCTG N0147 (Alliance)]

Frank A. Sinicrope[1], Qian Shi[2], Fabienne Hermitte[3], Erica Heying[2], Al B. Benson[4], Sharlene Gill[5], Richard Goldberg[6], Morton Kahlenberg[7], Suresh Nair[8], Anthony Shields[9], Dan Sargent[2], Jérôme Galon[10], Steven R. Alberts[1]

[1]Mayo Clinic, Rochester, MN, [2]Alliance Statistics and Data Center, Rochester, MN, [3]HalioDx, Marseille, France, [4]Northwestern Univ., Chicago, IL, [5]British Columbia Cancer Agency, Vancouver, BC, CAN, [6]Ohio State University Cancer Center, Columbus, OH, [7]Surgical Oncology Assoc. of South Texas, San Antonio, TX, [8]Lehigh Valley Hospital, Allentown, PA, [9]Karmanos Cancer Institute, Detroit, MI, [10]Laboratory of Integrative Cancer Immunology, INSERM, Paris, FR.

Background

Immunoscore was developed based on CD3⁺ and CD8⁺ T-cell density and location in primary CC pts with pooled stages and varying treatment and follow-up. We determined if individual immune markers and/or Immunoscore are prognostic in resected stage III CC pts from FOLFOX arm of a phase III trial.

Objectives

To determine if individual immune markers and/or the Immunoscore® are prognostic in resected stage III CC pts from FOLFOX arm of a phase III adjuvant trial.

Methods

PATIENTS:

- Stage III colon cancer patients (N=600) randomly selected from the FOLFOX alone arms enrolled in NCCTG N0147, a phase III randomized adjuvant trial of FOLFOX +/- cetuximab (NCT00079274).

IMMUNE MARKERS:

- Density of CD3⁺ and CD8⁺ T-cells, or CD20⁺ B lymphocytes, in central tumor (CT) and invasive margin (IM) was evaluated by IHC and quantified using image analysis software.
- Immunoscore® was calculated using densities of CD3⁺ and CD8⁺ in both CT and IM per algorithm developed on a cohort including stage II and III pts. Galon J, et al JCO 34, 2016 (abstr 3500)
  - High risk: Immunoscore of 0 and 1
  - Low risk: Immunoscore of 2, 3, and 4

STATISTICAL ANALYSIS:

- Primary outcome: Disease-free survival (DFS)
- Associations evaluated by Kaplan-Meier curves and multivariable Cox model adjusting for age, gender, race, T/N stage, PS, sidedness, *KRAS/BRAF*, MMR, BMI, and TILs.
- Model selection procedure (backwards selection) was used to identify most important immunomarker(s) in predicting DFS
- Contal and O'Quigley method, highest hazard ratio (HR) and lowest p-value, and False Discovery Rate were used to identify optimal cutoffs to define high vs. low risk patient groups based on identified immunomarker(s).
- Pre-defined cut-offs were used to validate the standardized Immunoscore®.

Results

Figure 1. Histograms of immunomarkers density (cells/mm2)

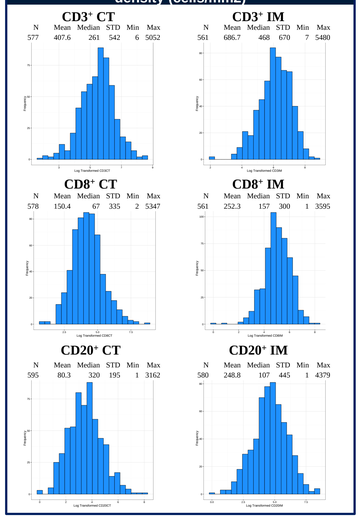


Table 1. Association between individual continuous immunomarker and DFS

Immunomarker*	HR_{adj} (95%CI)	p_{adj}
CD3CT	0.89 (0.79, 0.99)	0.033
CD3IM	0.89 (0.83, 0.96)	0.0018
CD8CT	0.79 (0.61, 1.02)	0.070
CD8IM	0.79 (0.65, 0.95)	0.013
CD20CT	0.92 (0.71, 1.17)	0.48
CD20IM	0.93 (0.85, 1.03)	0.16

*200 units increase

Model selection and risk group determination

Model Selection:

- When adjusted for known factors and after backwards selection, only CD3⁺ IM remained in the model, i.e. was most important immunomarker in predicting DFS.

Risk Group Determination:

- Density of 690 for CD3⁺ IM was identified as optimal cutoff to define high vs. low risk groups.
  - High risk: CD3⁺ IM < 690
  - Low risk: CD3⁺ IM ≥ 690

Table 2. CD3IM risk group and patient characteristics*

	<690 (N=368)	>=690 (N=193)	Total (N=561)	p value
Age				0.083
Median	58.0	60.0	59.0	
Range	(21.0-81.0)	(32.0-83.0)	(21.0-83.0)	
Gender				0.76
Female	180 (65.0%)	97 (35.0%)	277 (49.4%)	
Male	188 (66.2%)	96 (33.8%)	284 (50.6%)	
Race				0.64
Asian	19 (73.1%)	7 (26.9%)	26 (4.8%)	
Black	28 (62.2%)	17 (37.8%)	45 (8.3%)	
White	309 (65.3%)	164 (34.7%)	473 (86.9%)	
T Stage				<0.0001
T1 or T2	29 (39.2%)	45 (60.8%)	74 (13.2%)	
T3	295 (69.6%)	129 (30.4%)	424 (75.6%)	
T4	44 (69.8%)	19 (30.2%)	63 (11.2%)	
N Stage				0.0004
1-3	193 (59.6%)	131 (40.4%)	324 (57.8%)	
>=4	175 (73.8%)	62 (26.2%)	237 (42.2%)	
Tumor Location				0.010
Right	156 (60.2%)	103 (39.8%)	259 (46.7%)	
Left	209 (70.6%)	87 (29.4%)	296 (53.3%)	
Tils				0.0014
High Tils (>4)	24 (47.1%)	27 (52.9%)	51 (11.3%)	
Low Tils (<=4)	279 (69.4%)	123 (30.6%)	402 (88.7%)	
BRAF/KRAS (3 level)				0.85
wtkras/wtbraf	189 (64.3%)	105 (35.7%)	294 (54.2%)	
mutkras/wtbraf	115 (66.9%)	57 (33.1%)	172 (31.7%)	
wtkras/mutbraf	50 (65.8%)	26 (34.2%)	76 (14.0%)	
MMR				0.0017
pMMR	340 (68.1%)	159 (31.9%)	499 (89.7%)	
dMMR	27 (47.4%)	30 (52.6%)	57 (10.3%)	

*Only includes patients with nonmissing CD3⁺ IM values

Figure 2. CD3⁺ IM risk group and DFS**

Figure 2A. All patients

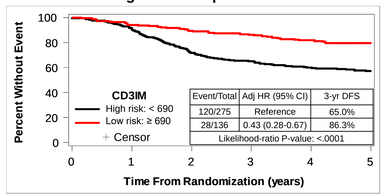


Figure 2B. Excluding dMMR patients

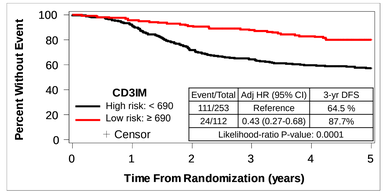


Figure 2C. CD3⁺ IM risk group and DFS per subgroup

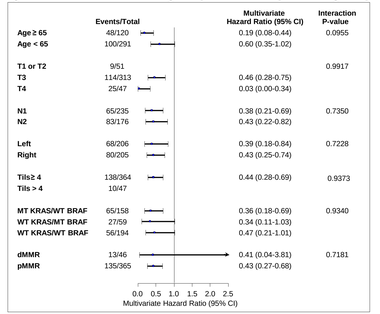


Figure 2. CD3⁺ IM risk group and DFS

Figure 3. Immunoscore® and DFS**

Figure 3A. All patients

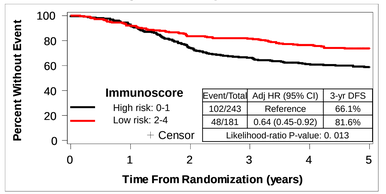


Figure 3B. Excluding dMMR patients

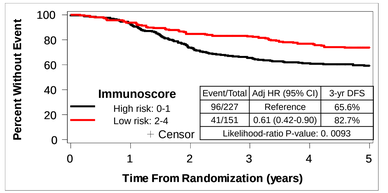
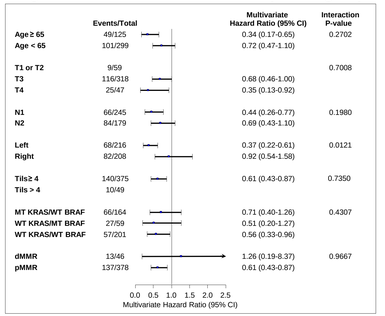


Figure 3C. Immunoscore ® and DFS per subgroup



**Each model has patients with complete data on immunomarker(s) and included covariates

Key Findings

- CD3⁺ IM (< 690)-defined high risk group was associated with $T_{3/4}$ (p<.0001), N_2 (p=0.0004), left-sided location (p=0.010), low TILs (p=0.0014), and proficient MMR (p=0.0017).
- Individually, higher density of CD3⁺ CT, CD3⁺ IM and CD8⁺ IM were significantly associated with longer DFS adjusting for covariates.
- CD3⁺ IM had the strongest association with DFS, and can be used to classify patients into low (≥690, better DFS) vs. high (<690, worse DFS) risk groups ($HR_{Adj.}$=0.46, 95% CI, 0.31-0.67, $p_{Adj.}$ <0.0001)
- Using a prior Immunoscore® risk stratification, higher scores were associated with significantly better DFS ($HR_{Adj.}$=0.62, 95% CI, 0.45-0.86, $p_{Adj.}$= 0.0030)
- Results remained very similar after excluding dMMR pts
- Risk classification based on CD3⁺ IM alone (c statistics, 0.70) provides as good of prediction accuracy as Immunoscore® (c statistics, 0.69). CD3⁺ IM risk prediction was consistent among subgroups.

Conclusions

- Densities of CD3⁺ and CD8⁺, especially at IM, were individually prognostic in FOLFOX-treated stage III pts.; CD3⁺ IM had strongest association with outcome.
- Immunoscore® (using pre-defined cutoffs) was strongly prognostic, and this result provides validation in a phase III clinical trial cohort
- In resected stage III CCs, risk classification based on immune marker(s) or Immunoscore® provides clinically useful prognostic stratification.

Supported by National Cancer Institue and National Institutes of Health grants: U10CA180821, U10CA180882, U10CA180820, U10CA180863, U10CA180888, CCSRI 021039