Xerna tumor microenvironment subtypes as a biomarker in lung cancer patients

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1. Introduction

Immune checkpoint inhibitor (ICI) monotherapy is guideline approved in NSCLC [1].

The predominant standard of care utilizes combinations of ICI with chemotherapy, or precision therapies targeting oncogenic drivers.

Biomarkers guiding these clinical decisions rely on tumor genotyping to identify targetable oncogenic drivers, high tumor mutational burden (TMB) as well as PD-L1 expression.

Currently, neither PD-L1 nor TMB perform adequately for ICI patient selection [2].

Emerging evidence indicates a more complete profile of the tumor microenvironment (TME) may improve selection of patients likely to respond to ICI [3].

The Xerna[TM] machine learning-based RNA sequencing biomarker assay classifies tumors into four TME subtypes (*Figure 1*):

I. **Angiogenic (A).**
II. **Immune Active (IA).**
III. **Immune Desert (ID).**
IV. **Immune Suppressed (IS).**

This classification identifies tumors likely to benefit from ICI (IA and IS) or anti-angiogenic agents (A and IS). ID tumors are predicted to likely benefit from agents that stimulate de novo immune responses or from those that specifically target tumor cell biological pathways, such as cell cycle inhibitors. [4]

We examined the distribution of actionable oncogenic driver mutations across Xerna TME subtypes to investigate the potential use for treatment decisions.

2. Methods

Biomarker prevalence, and Xerna TME subtype classification, were determined for 104 metastatic lung cancer cases previously submitted for Oncomap[TM] ExTra testing.

The Oncomap ExTra test uses tumor-normal, whole-exome and whole-transcriptome sequencing.

RNA expression levels from the whole-transcriptome sequencing were used to calculate Xerna scores and assign tumors to the four Xerna subtypes (*Figure 1*).

Somatic DNA variants and high TMB (≥10 mut/Mb) were identified from the DNA sequencing data.

Actionable alterations were defined based on their ability to predict response/resistance to targeted therapy in any cancer type, or NSCLC clinical trial eligibility, using a comprehensive and curated knowledge base.

Associations between somatic variants and Xerna subtypes were compared using Fisher's Exact Test.

The study was approved by WCG IRB Ethics Board, approval number 20181863.

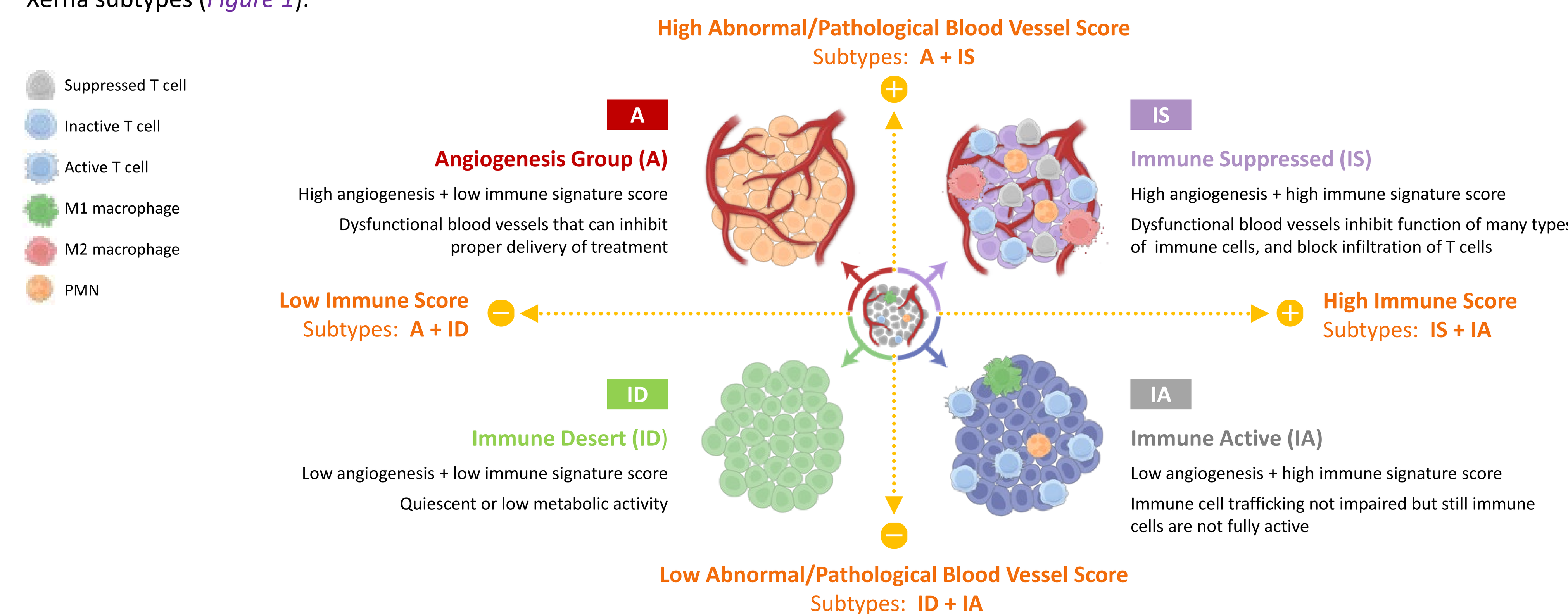

Figure 1: *The machine learning-based Xerna score is obtained from RNA gene expression levels of ~100 genes. The score reflects the dominant cellular microenvironment of the tumor, along immune and angiogenic axes, and may be useful for predicting response to particular therapies, thus informing therapy decisions.*

3. Results

The characteristics of the patient cohort and the distribution of Xerna subtypes are shown in *Table 1*. Most patients were either ID (n=34, 32.7%) or IS (n=42, 40.4%), with relatively few A (n=15, 14.4%) or IA (n=13, 12.5%).

Combining subtypes to focus on the immune environment axis, 55 (52.9%) samples had high (IA+IS) and 49 (47.1%) had low (ID+A) Xerna immune scores.

Gender frequencies within Xerna subtypes were not different (Fisher's Exact Test).

Patient samples harbored between 0 and 12 actionable alterations, 77 (74.0%) carried an actionable alteration associated with a targeted therapy, and 101 (97.1%) carried an actionable alteration associated with targeted therapy or clinical trial (*Table 2*).

A selection of genes and their associations with Xerna subtypes are shown in *Table 3*. Seven biomarkers (*B2M*, TMB high, *LRP1B*, *TP53*, *RB1, JAK2, CDKN2B*) showed significant associations with Xerna subtypes.

Of note, all but one of these biomarkers were more common in the Immune Active (IA) subtype. The exception was *CDK2NB*, which was most common in the Immune Desert (ID) subtype.

Analysis of biomarkers with high versus low Xerna immune scores revealed only one (*CDKN2B*) that was borderline significantly associated (p=0.05), with greater incidence (8.2% vs 0%) in the low immune score group.

Table 1: *Patient characteristics and Xerna tumor microenvironment subtype / immune group.*

		Xerna subtype				Immune group	
Variable	**All patients**	**A**	**IA**	**ID**	**IS**	**High (IA/IS)**	**Low (A/ID)**
No. of samples							
n	104	15 (14.4%)	13 (12.5%)	34 (32.7%)	42 (40.4%)	55 (52.9%)	49 (47.1%)
Age (years)							
Mean (SD)	65.9 (11.31)	66.2 (13.91)	61.5 (15.57)	64.0 (10.32)	68.6 (9.06)	66.9 (11.20)	64.7 (11.43)
Median	67.0	68.0	66.0	63.5	69.0	68.0	65.0
Q1-Q3	57.5 - 74	58 - 75	57 - 71	56 - 72	64 - 76	63 - 75	56 - 74
Min, Max	28, 92	38, 92	28, 81	47, 82	45, 83	28, 83	38, 92
Gender							
Female	58 (55.8%)	13 (86.7%)	5 (38.5%)	13 (38.2%)	27 (64.3%)	32 (58.2%)	26 (53.1%)
Male	46 (44.2%)	2 (13.3%)	8 (61.5%)	21 (61.8%)	15 (35.7%)	23 (41.8%)	23 (46.9%)
Actionable Mutations per Sample							
Mean (SD)	3.0 (2.08)	2.7 (1.72)	4.0 (3.29)	3.3 (2.23)	2.6 (1.45)	2.9 (2.09)	3.1 (2.09)
Median	2.5	3.0	3.0	2.5	2.0	2.0	3.0
Q1-Q3	2 - 4	1 - 4	2 - 5	2 - 5	2 - 3	2 - 3	2 - 4
Min, Max	0, 12	0, 6	0, 12	0, 10	1, 7	0, 12	0, 10

Table 2: *Number of samples with actionable alterations.*

TME Subtype/Group	No. Samples Analyzed (n=104)	Biomarker associated with targeted therapy* (n=77)	Biomarker associated with NSCLC clinical trial (n=90)	All actionable biomarkers (n=101)
A	15 (14.4%)	14 (93.3%)	9 (60.0%)	14 (93.3%)
IA	13 (12.5%)	10 (76.9%)	12 (92.3%)	12 (92.3%)
ID	34 (32.7%)	23 (67.6%)	31 (91.2%)	33 (97.1%)
IS	42 (40.4%)	30 (71.4%)	38 (90.5%)	42 (100.0%)
High Immune Score (IA/IS)	55 (52.9%)	40 (72.7%)	50 (90.9%)	54 (98.2%)
Low Immune Score (A/ID)	49 (47.1%)	37 (75.5%)	40 (81.6%)	47 (95.9%)

*predicted response/resistance to targeted therapy in any cancer

Table 3: *The number (%) of patient samples carrying selected biomarkers with actionable alterations across Xerna tumor microenvironment subtypes.*

		Xerna subtype				
Biomarker*	**Total**	**A (n=15)**	**IA (n=13)**	**ID (n=34)**	**IS (n=42)**	**P-value Exact Test**
B2M	3 (2.9%)	0 (0.0%)	3 (23.1%)	0 (0.0%)	0 (0.0%)	**0.002**
TMB high	28 (26.9%)	2 (13.3%)	8 (61.5%)	12 (35.3%)	6 (14.3%)	**0.004**
LRP1B	4 (3.8%)	0 (0.0%)	3 (23.1%)	1 (2.9%)	0 (0.0%)	**0.01**
TP53	57 (54.8%)	4 (26.7%)	10 (76.9%)	23 (67.6%)	20 (47.6%)	**0.02**
RB1	15 (14.4%)	0 (0.0%)	4 (30.8%)	8 (23.5%)	3 (7.1%)	**0.02**
JAK2	5 (4.8%)	0 (0.0%)	2 (15.4%)	3 (8.8%)	0 (0.0%)	**0.04**
CDKN2B	4 (3.8%)	0 (0.0%)	0 (0.0%)	4 (11.8%)	0 (0.0%)	**0.04**
KRAS	21 (20.2%)	2 (13.3%)	1 (7.7%)	4 (11.8%)	14 (33.3%)	0.07
ERBB2	3 (2.9%)	2 (13.3%)	0 (0.0%)	1 (2.9%)	0 (0.0%)	0.07
EGFR	32 (30.8%)	7 (46.7%)	2 (15.4%)	7 (20.6%)	16 (38.1%)	0.12
FGFR3	1 (1.0%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	0.13
MTAP	3 (2.9%)	0 (0.0%)	0 (0.0%)	3 (8.8%)	0 (0.0%)	0.13
STK11	8 (7.7%)	1 (6.7%)	3 (23.1%)	2 (5.9%)	2 (4.8%)	0.17
RET	2 (1.9%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	1 (2.4%)	0.35
CDKN2A	11 (10.6%)	1 (6.7%)	1 (7.7%)	6 (17.6%)	3 (7.1%)	0.51
PTEN	7 (6.7%)	1 (6.7%)	2 (15.4%)	2 (5.9%)	2 (4.8%)	0.56
MET	2 (1.9%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	1 (2.4%)	0.57
APC	4 (3.8%)	0 (0.0%)	1 (7.7%)	1 (2.9%)	2 (4.8%)	0.71
MDM2	5 (4.8%)	1 (6.7%)	1 (7.7%)	1 (2.9%)	2 (4.8%)	0.72
BRAF	3 (2.9%)	0 (0.0%)	0 (0.0%)	2 (5.9%)	1 (2.4%)	0.84
PIK3CA	9 (8.7%)	1 (6.7%)	1 (7.7%)	3 (8.8%)	4 (9.5%)	1
FGFR1	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.4%)	1

*Genes with alterations not shown in table (none showed significant associations): *APC, ARID1A, ATM, CSMD3, EP300, NF1, PTPRD, RASA1, YEATS4*.
Xerna tumor microenvironment subtypes: A= Angiogenic, IA=Immune Active, ID=Immune Desert, IS=Immune Suppressed

4. Conclusions

The Xerna TME panel identified a high prevalence of patients who may benefit from ICI (IA/IS), most of whom also carried an actionable biomarker identified by the Oncomap Extra assay.

The prevalence of targetable oncogenic drivers within the IS subtype, such as *KRAS* G12C, may represent the potential for novel KRAS G12C inhibitors with ICI combination therapies [5].

Alterations of *CDKN2A*, *CDKN2B* and *MTAP* genes located on chr 9p21 were highest in ID vs other subtypes (though not significantly different for *CDKN2A* and *MTAP*) suggesting a "cold" tumor-immune phenotype with activation of immunosuppressive signaling [6]. Such tumors may be candidates for cell-cycle inhibitors or stimulators of *de novo* immune responses (e.g., tumor vaccines).

Mutations in *LRP1B*, which are associated with preferable clinical outcome in ICI therapy, as well as loss of function *B2M* and *JAK2* mutations associated with acquired resistance to cancer immunotherapy, were found to be highest in IA versus the other 3 subtypes. Information provided by the combined Oncomap ExTra/Xerna TME panel profiling thus gives a more robust assessment of candidacy for ICI treatment in IA tumors.

TMB-high was seen in all Xerna subtypes, including those with low immune scores (A/ID), suggesting some TMB-high patients may be unlikely to respond to ICI treatment because of the TME.

Findings highlight the value of adding TME analysis to comprehensive biomarker testing in NSCLC.

5. References

1. National Comprehensive Cancer Network. Non-small cell cancer v.5.2022. https://www.nccn.org/guidelines. Accessed 10/20/2022.
2. Steuer CE, Ramalingam SS. *JCO Oncol Pract*. 2021 doi: 10.1200/OP.21.00305.
3. Horvath L, et al. *Mol Cancer*. 2020. doi: 10.1186/s12943-020-01260-z.
4. Iyer, S., et al. *Cancer Research* 2022. (abstract) doi: 10.1158/1538-7445.AM2022-1232.
5. Mugarza E, et al. *Sci Adv*. 2022 doi: 10.1126/sciadv.abm8780.
6. Han, G, et al. *Nat Commun.* 2021. doi: 10.1038/s41467-021-25894-9.

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