Xerna™ TME Panel: A pan-cancer RNA-based investigational assay designed to predict patient response to angiogenic- and immune-targeted therapies

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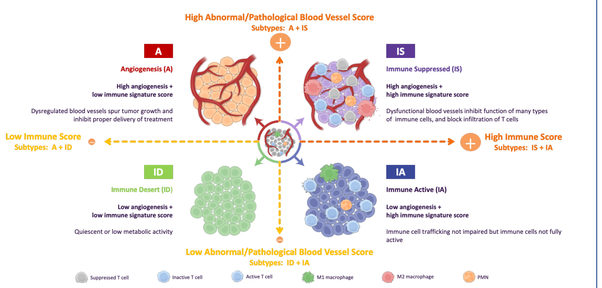
Abstract

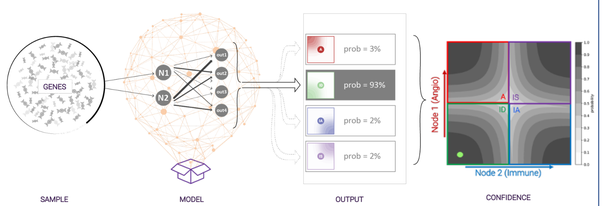
While numerous anti-angiogenic and immune targeting therapies have become standard-of-care treatments for oncology, predictive biomarkers for these agents have been either entirely lacking or challenged by inconsistencies across indications. We have developed and validated the Xerna TME Panel as a novel machine learning-based RNA-sequencing biomarker assay that guides patient selection for tumor microenvironment (TME)-targeted therapies across multiple tumor types. Gene expression data sets from both public sources and clinical practice representing over 5000 samples across 7 different tumor types were analyzed using the Xerna TME Panel. The Xerna TME Panel consists of an artificial neural net that learns complex gene expression interactions between angiogenesis and tumor immune biologies and robustly classifies patient samples into one of four TME biomarker subtypes: Angiogenesis (A), Immune Active (IA), Immune Desert (ID), or Immune Suppressed (IS). The vast majority (>75%) of all samples were assigned a TME class designation with confidence scores in the upper quartile and had nearly bimodal distributions for biomarker-positive versus -negative classifications. When compared to other independent gene signatures, such as those describing angiogenesis/mesenchymal biology, inflammation, and immune suppression, the expression profiles from the Xerna TME subtypes showed enrichment of those biological processes. Each TME subtype represented between ~15-40% of subjects of each tumor type, indicating balanced representation of subgroups within the patient populations. The Xerna TME designations were prognostic across tumor types, with “A” tumors generally associated with the worst survival and “IA” tumors associated with the best survival. The predictive ability of the Xerna TME Panel to enrich for tumor responses to targeted therapies in gastric cancer was also evaluated. In a ramucirumab+paclitaxel clinical cohort, the Xerna TME Panel high Angiogenesis score tumors (A and IS) demonstrated a 50% response rate compared to a 31% for low Angiogenesis score tumors (IA and ID). In an immune checkpoint inhibitor (ICI) cohort, high Immune score tumors (IA and IS) showed a response rate of 34% vs. 5% for low Immune score tumors (A and ID). Within the microsatellite stable patients (MSS), which historically have low response rates to ICIs, the Xerna TME Panel was able to enrich for responses between Immune high vs. Immune low score patients (25% vs. 3%). Currently in use to prospectively enroll patients into a Phase 3 ovarian cancer clinical trial and in development as a companion diagnostic (CDx) assay, the Xerna TME Panel is a robust, pan-cancer biomarker assay capable of characterizing TME dominant biologies to further advance the matching of patients with targeted therapeutics.

Overview of Xerna TME Panel



The Xerna TME Panel is a novel diagnostic assay that uses formalin-fixed paraffin-embedded (FFPE) tissue-derived RNA gene expression data based on ~100 genes to classify patients into dominant biologies of the tumor microenvironment (TME). The input gene signature represents angiogenic and immunogenic properties of stromal biology, and the machine learning neural network that comprises the Xerna TME Panel algorithm has learned interactions between these critical processes. The Xerna TME Panel can be used to classify a patient’s tumor microenvironment along an immune and angiogenic axis, resulting in one of four TME phenotypes—Angiogenic (A), Immune Active (IA), Immune Desert (ID), and Immune Suppressed (IS) (figure above). Each class, or combinations of classes, can be predictive of treatment outcome with various targeted therapies, including anti-angiogenics and checkpoint inhibitors, as well as novel drugs targeting the intersection of these biologies.

Xerna TME Panel Workflow



Gene expression data (SAMPLE) is analyzed through a machine learning-based artificial neural net algorithm (MODEL) which provides a single subtype designation (OUTPUT) that is based on probabilized scores of the TME subtypes (CONFIDENCE). Shown here is a representation of a single tissue sample analyzed by the model with its subtype designation “ID” and visualization on a latent space plot highlighted as a green circle.

Patient Sample Cohorts Used for Development and Testing

	Tumor Type	Independent Cohorts		Xerna TME Panel Successfully Predicted Outcomes
Training:	Gastric	ACRG, no targeted therapy, comparable clinical history[1]	N=298	N/A
	Multiple Solid Tumors	Misc. Biobanked Samples[2]	N= ~1,100	N/A
		Gene List and Algorithm Locked		
Testing:	Gastric/GEJ	Immune checkpoint inhibitor[3] Anti-angiogenic + Chemo[4] Combo Immune Therapy[5]	N=73 N=49 N=57	✔ ✔ ✔
	Ovarian	Anti-angiogenic + Chemo[6]	N=33	✔
	Colorectal	CIT Stage 0-2[7]	N=557	✔
	Multiple Solid Tumors	Other clinical collections and public sources, multiple indications[8]	N > 3100	Ongoing
Explanation:	TCGA-STAD	Real world data (RWD), no targeted therapy[9]	N=388	N/A
	Singapore Gastric	RWD, mixed lines of treatment[10]	N=192	N/A

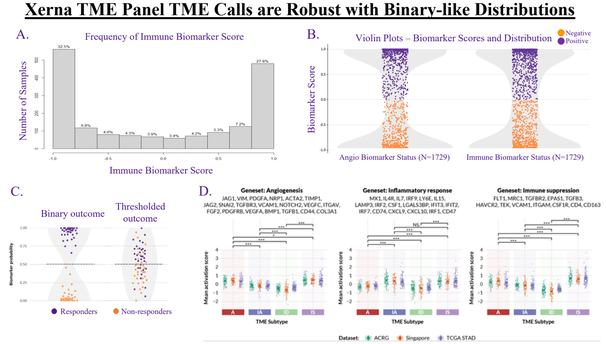
[1] Asian Cancer Research Group (ACRG); publicly available data from Cristescu et al, Nature Medicine 2015
[2] HTG Molecular Diagnostic Inc.; samples purchased - Gastric (N=337), colorectal (N=370), and ovarian (N=392)
[3] Samsung Medical Center; samples from clinical practice
[4] Samsung Medical Center; samples from clinical practice
[5] ONCG100 clinical trial (NCT04099641); samples from OncXerna sponsored trial
[6] Nerviani clinical trial (NCT03038087); samples from OncXerna sponsored trial
[7] Curtes d’Identite des Tumeurs (CIT); publicly available from Marisa et al, PLOS Medicine 2013
[8] Publicly available ovarian, breast, colon, rectal, prostate, liver, gastric, and melanoma data from The Cancer Genomic Atlas Program (TCGA) and collaboration samples from Moffitt Cancer Center
[9] The Cancer Genome Atlas (TCGA) gastric cancer cohort; publicly available from TCGA, Nature 2014
[10] Real world gastric cancer patient samples publicly available from Liu et al, Gastroenterology 2013.

Analysis of Tumor Samples - TME Biomarker Distributions

Metastatic Cancer Types	Number of Samples Analyzed	Samples with TME Call Confidence >75% (%)	Angiogenesis (A) Subtype (%)	Immune Active (IA) Subtype (%)	Immune Desert (ID) Subtype (%)	Immune Suppressed (IS) Subtype (%)	Angiogenesis Biomarker Positive – A + IS (%)	Immune Biomarker Positive – IA + IS (%)
Ovarian	804	81.6	18.8	18.4	34.6	28.2	45.8	46.7
Breast	424	81.6	25.5	20.5	26.9	27.1	52.6	47.6
Colorectal	1854	82.7	17.2	18.0	34.7	30.2	47.4	48.1
Prostate	499	83.2	20.2	12.4	33.5	33.9	54.1	46.3
Liver	374	87.7	14.2	14.7	41.4	29.7	43.9	44.4
Gastric	891	81.6	21.0	23.2	30.1	25.7	46.7	49.0
Melanoma	471	83.0	20.2	21.2	32.3	26.3	46.5	47.6

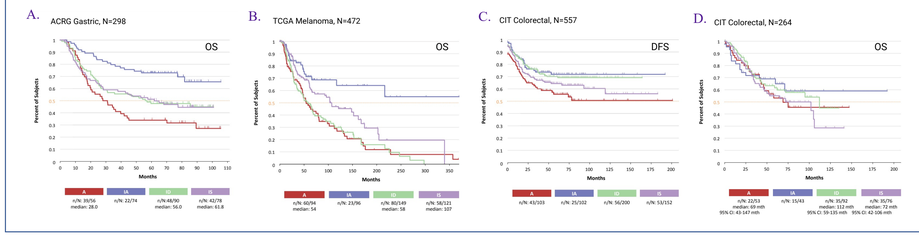
Xerna TME calls were assessed for over 5,300 samples across 7 different tumor types. Individual calls were one of four subtypes: A (angiogenesis), IA (Immune active), ID (immune desert), or IS (immune suppressed). Each call was based upon its highest probability (confidence) TME subtype. TME subtypes were also combined into groups to represent the two primary axes of Angiogenesis (A + IS) and Immune (IA +IS) biologies. Non-Angio and Non-Immune group is represented by the ID subtype. A vast majority of samples have TME calls with greater than 75% confidence. While the prevalence of Immune and Angiogenesis dominant biology varies across different tumors there is a high representation of both Angio and Immune dominant biology in all tumor types.

Xerna TME Panel TME Calls are Robust with Binary-like Distributions



A. Immune biomarker scores from a subset of the cohorts (N=1729 samples) were graphed according to their distribution. Scores range from -1.0 to 1.0 with negative scores represented in the A and ID groups and positive scores represented in the IA and IS groups. **B.** Violin plots for both the Angio and Immune Biomarker scores across multiple combined cohorts (N=1729) demonstrate the binary-like distribution of biomarker designations. **C.** Schematic of the output distribution of the Xerna TME panel contrasts with most current biomarker assay outcomes that are near-normal in their distributions and often cannot define clear thresholds for positive and negative populations. **D.** Activation scores (y-axis) were computed on patient samples from the ACRG, Singapore Cohort, and TCGA-STAD (stomach adenocarcinoma) datasets for gene sets representing angiogenesis and mesenchymal biology, inflammatory response and immune suppression. The gene sets were manually derived subsets of the GSEA MSigDB Hallmark collections and are listed above each plot. In each plot, the datasets are colored according to the legend and grouped by TME subtypes A, IA, ID and IS. All pairwise comparisons between TME subgroups were analyzed for statistical differences in mean activation score with (*) indicating p-value < 0.05, (***) indicating p-value < 0.0005, and “NS” indicating no significant difference.

Xerna TME Panel TME Calls are Prognostic Across Multiple Tumor Types and Patient Cohorts



A. Kaplan-Meier (KM) plot of overall survival in the ACRG gastric cohort (N=298; 2L patients receiving chemo and/or radiation) grouped by TME subtype.

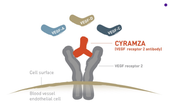
B. KM plot of overall survival in the TCGA melanoma cohort (N=472; primary or metastatic patients with no prior systemic therapy) grouped by TME subtype.

C. KM plot of disease-free survival in the CIT colorectal cohort (N=557; Stage 0-4 patients who had curative surgery) grouped by TME subtype.

D. KM plot of overall survival in the CIT colorectal cohort (N=264; Stage 3-4 patients who had curative surgery) grouped by TME subtype.

Predictive Capability of Xerna TME Panel for Anti-Angiogenic Therapy

CYRAMZA™ (ramucirumab)

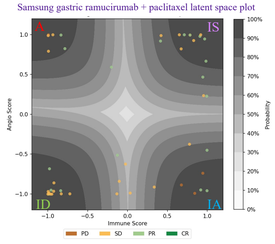


- >3rd line gastric cancer patients treated at Samsung Medical Center with a combination of ramucirumab (anti-VEGFR2) and paclitaxel (N=49 with biomarker data; N=48 with clinical response data)
- ORR and PFS data was available for assessment of biomarker predictive potential

Tumor samples were collected as surgical resections prior to systemic therapy
- Samples were formalin-fixed paraffin-embedded (FFPE)
- RNA was extracted and RNA-Seq run
- Analyzed in the Xerna TME panel

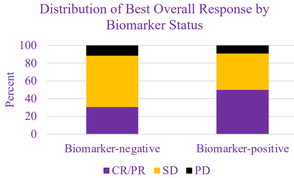
Hypothesis for ramucirumab + paclitaxel treatment is the TME Angio subtypes (A and IS) were more likely to derive the most clinical benefit.

Samsung gastric ramucirumab + paclitaxel latent space plot



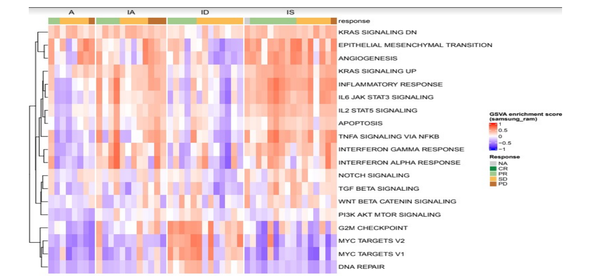
Latent space plot of Xerna TME calls for samples from the Samsung gastric ramucirumab + paclitaxel cohort. Glyphs are color-coded according to their best response to ramucirumab + paclitaxel. Contours represent different levels of probabilities for the Xerna TME calls.

Distribution of Best Overall Response by Biomarker Status



Best Overall Response by Xerna TME Biomarker Status

TME call/group	Number	CR/PR	SD	PD	ORR (%)
All Patients	48	19	24	5	39.58
A	7	3	3	1	42.86
IS	15	8	6	1	53.33
IA	9	3	3	3	33.33
ID	17	5	12	0	29.41
Angio (A+IS)	22	11	9	2	50.00
Non-Angio (IA+ID)	26	8	15	3	30.77



Heatmap of Gene Set Variation Analysis (GSVA) signature scores clustered by Xerna TME subtypes. GSVA shows enrichment of various pathways (rows) for each patient in the Samsung gastric ramucirumab + paclitaxel cohort (columns). Patients are grouped by TME subtype and tumor response based on RECIST criteria, shown according to the legend.

Biomarker Performance Characteristics – Ramucirumab Gastric Cohort

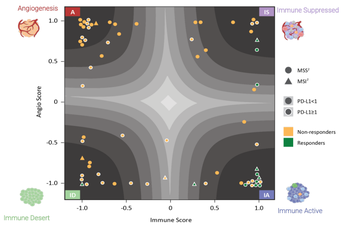
Biomarker Positive	ACC	Sensitivity	Specificity	PPV	NPV
Angio Biomarker-positive (A + IS subtypes)	0.60 (29/48)	0.58 (11/19)	0.62 (18/29)	0.50 (11/22)	0.69 (18/26)
Random	0.53 ± 0.064	0.40 ± 0.081	0.61 ± 0.053	0.40 ± 0.081	0.61 ± 0.053

ACC (accuracy): number of correct predictions /total number of predictions
Sensitivity: true biomarker responses / total actual responses
Specificity: true biomarker non-responses / total actual non-responses
PPV (positive predictive value): true biomarker responses / total predicted biomarker responses
NPV (negative predictive value): true biomarker non-responses/ total predicted biomarker non-responses

Predictive Capability of Xerna TME Panel for Immune Checkpoint Therapy

OPDIVO® (nivolumab)
KEYTRUDA® (pembrolizumab)

- 2nd and 3rd line gastric cancer patients treated at Samsung Medical Center with monotherapy pembrolizumab or nivolumab (N=73)
- ORR and PFS data was available for assessment of biomarker predictive potential
- MSI/MSS status and PD-L1 IHC CPS score determined for almost all patients
- Tumor biopsies were collected just prior to initiating ICI therapy
  - Samples were formalin-fixed paraffin-embedded (FFPE)
  - RNA was extracted and RNA-Seq run
  - Analyzed in the Xerna TME panel
- *TME High Immune subtypes (IA and IS) were hypothesized to derive the most clinical benefit.*



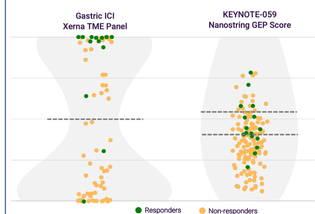
Latent space plot of Xerna TME calls for samples from the Samsung gastric ICI cohort. Glyphs are shaped according to their MSS/MSI status, outlined according to their PD-L1 CPS score status, and color-coded according to their best response. Contours represent different levels of probabilities for the Xerna TME calls.

Gastric cancer patient cohort (N=73) treated with immune checkpoint inhibitor (ICI) monotherapy

Best overall response rates comparison (%)

High probability IA subtype	PD-L1 positive
58%	30%
MSS + IA subtype	MSS all-comers
33%	12%
MSS + PD-L1 + Immune High subtypes	MSS + PD-L1 + Immune Low subtypes
44%	0%
MSI-H Immune High subtypes	MSI-H Immune Low subtypes
100%	25%

Table of best clinical response for select subtypes in the Samsung gastric cohort treated with ICI monotherapy. Overall response rate in the entire cohort is 17.8%. “High probability” IA subtype samples include those samples with IA score probabilities of 0.9 or higher.



Violin plots for the Samsung gastric cohort treated with ICI monotherapy (left) and for the Keynote-059 gastric cohort treated with pembrolizumab (right). The Keynote-059 trial was composed of gastric cancer patients with similar disease background as the Samsung cohort and utilized an 18 gene T-Cell-inflamed gene expression score from Nanostring (Fuchs et al, JAMA Oncology 2018). Xerna TME panel outputs in this cohort are binary-like and highly enriched for responders in the biomarker-positive group (above the dotted line). In the Keynote-059 cohort, the data has a normal distribution with a majority of data centered around the mean, creating a difficulty in assigning a threshold for biomarker status and discriminating responders from non-responders. RECIST criteria was used for clinical assessments for each of these cohorts.

Biomarker Performance Characteristics – Immune Checkpoint Inhibitor Gastric Cohort

Biomarker Positive	ACC	Sensitivity	Specificity	PPV	NPV
Xerna TME Panel: IA ≥90% Probability	0.85 (62/73)	0.54 (7/13)	0.92 (55/60)	0.58 (7/12)	0.90 (55/61)
Xerna TME Panel: IA+IS	0.68 (50/73)	0.85 (11/13)	0.65 (39/60)	0.34 (11/32)	0.95 (39/41)
PD-L1 CPS ≥1	0.59 (41/69)	1.00 (12/12)	0.51 (29/57)	0.30 (12/40)	1.00 (29/29)
MSI-H	0.85 (62/73)	0.38 (5/13)	0.95 (57/60)	0.63 (5/8)	0.88 (57/65)

ACC (accuracy): number of correct predictions /total number of predictions
Sensitivity: true biomarker responses / total actual responses
Specificity: true biomarker non-responses / total actual non-responses
PPV (positive predictive value): true biomarker responses / total predicted biomarker responses
NPV (negative predictive value): true biomarker non-responses/ total predicted biomarker non-responses

Summary and Conclusions

- **The Xerna TME Panel is a machine-learning based, artificial neural net model** using RNA-Seq technology for RNA derived from FFPE tumor tissue.
- Identifies the **dominant biology** of the tumor microenvironment and assigns into **therapeutically actionable tumor subtypes** defined by angiogenesis and immune gene expression.
- Model was **trained on biology**, **validated on multiple clinical cohorts**, and **tested for prediction to drug response** across hundreds of samples from **multiple different tumor types**.
- ANN algorithm outputs have **robust and binary-like distributions**, allowing for **high confidence biomarker calls**.
- TME subtypes have **varying prevalence** across tumor types, have **prognostic value** for determining survival and disease recurrence risk, and are **predictive for response** to anti-angiogenic and immune targeting therapies.
- Xerna TME Panel has been validated as a **CTA** for the upcoming REVELARE trial (Ph3 Ovarian for Navicixizumab) and is in development as an **RUO and CDx** .