# Radiomics of Metastatic Lesions in Gastroesophageal Adenocarcinoma (GEA) May Correlate with Tumoral DKK1 mRNA Expression and Other Immune Biomarkers in Patients Treated with DKN-01

Cynthia Sirard[1], Girish S. Naik[1], Michael Kagey[1], Samuel J. Klempner[2], Zak Nolte[3], Ziad Hindosh[3], Andre Burkett[3], Ronald Korn[3]

[1]Leap Therapeutics, Cambridge, Massachusetts, [2]Massachusetts General Hospital Cancer Center; Boston, MA; [3]Imaging Endpoints Research and Core Lab, Scottsdale, Arizona

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## BACKGROUND

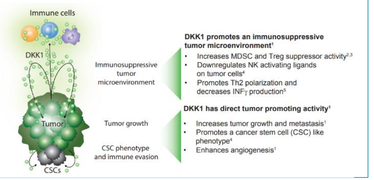
### Dickkopf-1 (DKK1)

- Modulator of Wnt signaling
- Mutations in Wnt activating genes (stabilizing β-catenin mutation; e.g., CTNNB1, APC and RNF43) lead to increased DKK1 expression
- Tumor cells secrete DKK1; elevated DKK1 expression = poor prognosis
  - → Immunosuppressive tumor microenvironment
  - → Promotes proliferation, metastasis, and angiogenesis

### DKN-01

- Humanized monoclonal antibody [IgG4] targeting DKK1
- Activates innate immune response in preclinical models
- Tumors with Wnt activating mutations are more likely to responded to DKN-01
- In esophagogastric cancer patients treated with DKN-01 + pembrolizumab, high tumoral DKK1 was associated with better response and survival (see poster #357)

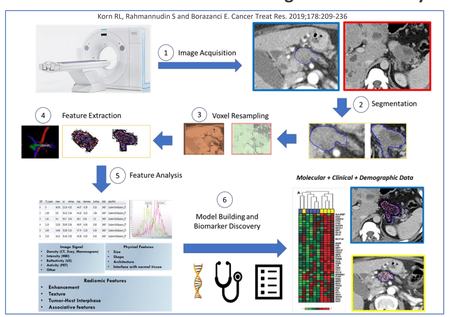
### Model for DKK1 Cancer-Promoting Activity[1-5]




### Radiomic Quantitative Texture Analysis (QTA)

- Non-invasive tool for imaging biomarker discovery on standard of care (SOC) CT, MRI and/or PET images
- Detects subtle but quantifiable changes in the signal intensities (texture) of diseased tissues and tumors
- QTA used alone or in combination with other biomarkers can create "radiophenotypic" signatures that are associated with molecular drivers (hallmarks) of cancer
- QTA may be a useful tool for predicting DKK1 expression and other relevant biomarkers in metastatic lesions from GEA patients using baseline scans

### General Workflow of Radiomic Signature Discovery




## HYPOTHESIS

Radiomic QTA can be used to identify DKK1 expression and other relevant biomarkers in metastatic lesions from GEA patients treated with DKN-01.

## METHODS

**59 Patients with Esophagogastric Cancer**

- Enrolled in Phase I/II trial of DKN-01 + pembrolizumab
- 53/59 Subjects had scans amenable for QTA analysis
- 48 male, 5 female
- Age range from evaluable lesions: 31-87 years

**RNA Scope H-Scores**

High vs low DKK1 RNA Scope H-scores were identified during the trial based on tertile analysis.

H-Score ≥35 (DKK1 high): n=20

H-Score <35 (DKK1 low): n=33

**Radiomic QTA**

Metastatic target lesions from baseline CTs with IV contrast underwent QTA analysis (TexRAD, CE mark, Essex, UK) after RECIST analysis.

ROIs were placed on the target lesions and histogram frequency curves (HFC) of the metastatic tumor pixels were generated.

**Statistical Analysis**

First order tumor HFC statistics (mean, SD, skewness, kurtosis, MPP) were correlated* with:

- Tumoral DKK1 mRNA expression
- Baseline plasma DKK1 levels
- Tumor type

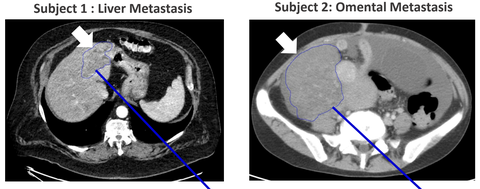
Lesion analysis included QTA from largest metastatic lesion and average QTA from target lesions on baseline scans.

*Pearson's r correlation, student's T-test (significance P < 0.05)

### Example QTA Analysis from Two Subjects with High (Subject 1) and Low (Subject 2) RNAscope H-scores for DKK1 Expression

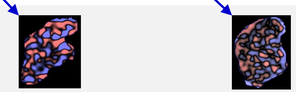
#### Axial Slices from Contrast-enhanced CT Scans of the Abdomen

Blue lines show the regions of interest drawn around target lesions using QTA software platform (TexRad, Essex, England)



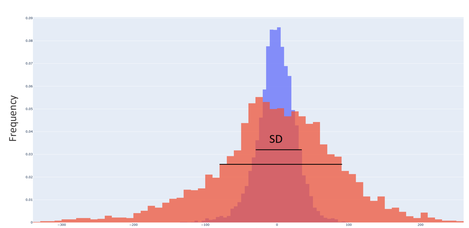

#### Voxel Resampling at Spatial Filter (SSF) 4

Transformed display of target lesion following QTA. Red, blue, and black highlights show range of pixel values from high density to low density, respectively.



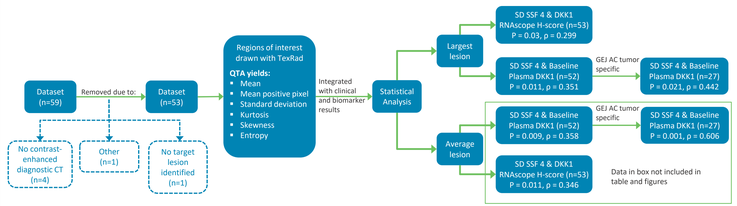
#### Histogram Frequency Curve (HFS) with High (≥35) and Low (<35) RNAscope Scores

The HFC show the frequency of pixel densities within tumor lesions (y-axis) vs pixel density (Hounsfield units, x-axis). An example of the HFCs showing the standard deviation (SD; black bars) from two subjects with high RNAscope H-scores (red HFC; H-score = 87) and low H-scores (blue HFC; H-score = 2) is shown here. Other first statistical values derived from the HFCs (not shown) include average pixel value, mean positive pixel value, entropy, skewness and kurtosis.



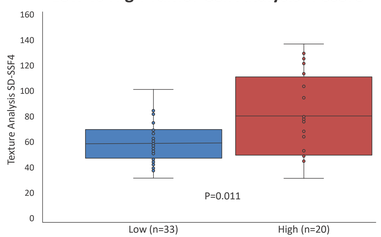

## RESULTS

### Summary Flow Chart of Results of Statistical Significance



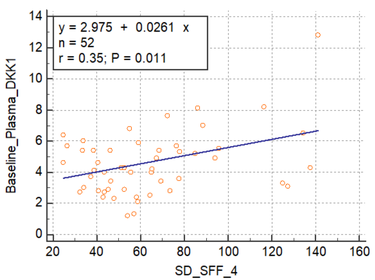

### Higher SD Values Are Associated With Higher Expression of DKK1

#### Low vs High Tumor Cell Analysis H-score




Box and whisker plots of SD(SSF4) values in subjects with low vs high RNAscope H-scores for DKK1 with mean SD values of 60.3 vs 79.2, respectively.

### Higher SD Is Correlated With Higher Plasma DKK1 Levels




Linear correlation (Pearson's) between baseline plasma DKK1 levels and SD(SSF4). Note that the higher SD values are associated with more DKK1 plasma level.

### Correlation between QTA and Plasma DKK1 Levels and H-scores for DKK1 Expression in Anti-PD1/PD-L1 Naïve vs Refractory Subjects in All Subjects (Largest Lesion) and Those with GEJ and GC AC

| QTA Variable= SD (SSF4) | Baseline Plasma DKK1* | | | RNA scope H-score | | |
|---|---|---|---|---|---|---|
| | r | P-Value | (Mean, SD) | r | P-Value | (Mean, SD) |
| Anti-PD1/PD-L1 Naïve | | n = 45 | | | n = 46 | |
| | 0.152 | 0.319 | (4.5, 1.9) | 0.336 | 0.022 | (49, 58.6) |
| Anti-PD1/PD-L1 Refractory | | n = 7 | | | n = 7 | |
| | 0.954 | 0.001 | (5.8, 3.7) | 0.143 | 0.759 | (23, 31.5) |
| Anti-PD1/PD-L1 Naïve + Refractory | | n = 52 | | | n = 53 | |
| | 0.351 | 0.011 | (4.7, 2.2) | 0.299 | 0.03 | (45.6, 56.3) |
| **Tumor Type: GEJ AC** | **Baseline Plasma DKK1*** | | | **RNA scope H-score** | | |
| QTA Variable= SD (SSF4) | r | P-Value | (Mean, SD) | r | P-Value | (Mean, SD) |
| Anti-PD1/PD-L1 Naïve | | n = 23 | | | n = 24 | |
| | 0.408 | 0.053 | (3.92, 1.5) | 0.26 | 0.219 | (52, 60.4) |
| Anti-PD1/PD-L1 Naïve + Refractory | | n = 27 | | | n = 28 | |
| | 0.442 | 0.021 | (4.1, 1.7) | 0.296 | 0.126 | (50.3, 57) |
| **Tumor Type: GC AC** | **Baseline Plasma DKK1** | | | **RNA scope H-score** | | |
| QTA Variable= SD (SSF4) | r | P-Value | (Mean, SD) | r | P-Value | (Mean, SD) |
| Anti-PD1/PD-L1 Naïve | | n = 5 | | | n = 5 | |
| | -0.335 | 0.582 | (3.6, 1.9) | 0.962 | 0.009 | (32, 61.3) |

*One Anti-PD1/PD-L1 Naïve patient missing baseline plasma DKK1 data

The table highlights significant (green box) and near significant (salmon box) correlations between SD(SSF4) and plasma DKK1 levels in all subjects, especially in anti-PD1/PD-L1 refractory compared to anti-PD1/PD-L1 naïve subjects. When tumor type is taken into account, there are significant associations between QTA and plasma DKK1 levels with GEJ AC but not GC AC subjects. Furthermore, the significant association between RNAscope H-scores and SD(SSF4) by QTA is seen in all subjects, especially treatment naïve subjects with GE AC. There were no additional significant correlations between PD-L1 expression, tumor shrinkage or Myeloid Derived Suppression Cell counts and QTA (data not shown).

## CONCLUSIONS

- Radiomics using QTA has identified SD(SSF4) of the HFC as a potentially useful biomarker to non-invasively evaluate tumoral DKK1 expression
- QTA analysis also demonstrates a significant correlation between SD(SSF4) and plasma DKK1 levels
- Tumor type and treatment status may influence these radiomic features with the strongest association between QTA and DKK1 expression seen in anti-PD1/PD-L1 naïve subjects with GC AC
- Although preliminary analysis suggested that QTA could distinguish tumors with PD-L1 high vs low expression, additional analysis did not confirm this finding (not shown)
- Further studies are warranted to determine if the SD radiomic signatures from QTA may provide an alternate non-invasive method for evaluating subjects with low vs high expression of DKK1 on SOC images in this population

## REFERENCES

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