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89Zr-Trastuzumab-PET, Rapid Autopsies, and Patient-Derived Xenografts to Determine the Extent of Clonal Evolution in Treatment-Refractory HER2+ Gastric Cancer

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14. ABSTRACT

Trastuzumab, an FDA approved monoclonal antibody that binds to the extracellular domain of HER2, is the standard of care for these patients. However, after an initial response secondary resistance invariably ensues. The molecular basis of therapeutic resistance in HER2-positive EG cancer remains unknown and, until now, no other HER2-directed therapy has shown clinical efficacy in patients with trastuzumab refractory EG cancer.

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INTRODUCTION

An estimated 1.5 million people were diagnosed with esophagogastric (EG) cancer worldwide in 2018, representing the third leading cause of cancer-related death.1 Moreover, the incidence of gastroesophageal junction (GEJ) cancer is increasing rapidly in Western countries, in particular among young individuals. Genomically, roughly a third of EG tumors are characterized by chromosomal instability and aberrant activation of human epidermal growth factor receptor (HER) tyrosine kinases (HER1-4). In patients whose cancers harbor ERBB2 amplification or overexpression of HER2 protein, the combination of the anti-HER2 antibody trastuzumab with cytotoxic chemotherapy (a fluoropyrimidine and platinum) is the recommended first-line therapy. Patients treated with this combination have a median overall survival (OS) of 13·8 months and an overall response rate (ORR) of 47%. While trastuzumab improves survival in patients with *ERBB2*-amplified cancer, disease progression still usually occurs within a year. Our ongoing Phase II of afatinib/trastuzumab and rapid autopsy program provided a unique opportunity to define the extent of genomic heterogeneity and tumor evolution upon pharmacological pressure in patients with trastuzumab-refractory, *ERBB2* amplified cancer.

We established an extensively characterized library of patient derived xenografts (PDX) from patients with HER2+ EG cancer. During the funding period of the award we sequenced and genomically characterized these PDXs and used en-vivo selection techniques to derive tumors with liver funded though Also, we will generate highly metastatic/aggressive derivative from the PDX. We will characterize these PDX to find out potential target pathways.

KEYWORDS
Trastuzumab, Rapid Autopsies, Patient Derived Xenografts, PDXs, Functional PET,
HER2+ gastric cancer

ACCOMPLISHMENTS

What are the major goals of the project?

1. To develop molecular targeted PET scan for patients with HER2+ EG cancer
2. Collection and analysis of HER2+ tumor samples from patients enrolled in phase II trial
3. To develop hypothesis driven combination strategies to overcome trastuzumab resistance in HER2+ EG cancer
4. Accrue patients with HER2+ EG cancer to MSK phase II clinical trials
5. Continue to establish EG cancer patient derived xenograft in immune deficient mice to reflect wide variety of patients
6. In-vivo selection of metastatic PDXs to develop models with propensity to develop liver metastasis

What was accomplished under these goals?

1. Regulatory approval of MSKCC IRB 13-165 by HRPO in May 2017 (Janjigian)
2. Regulatory approval of MSKCC IRB 11-116 by HRPO in June 2017 (Janjigian)
3. Specific Aim 1

    Major Task 1-

    a. ^{89}Zr-trastuzumab radiotracer manufacturing (Lewis) - Zr-trastuzumab manufacturing is a success and ongoing.

b. Perform ^{89}Zr-trastuzumab PET/CTs pre and post HER2 directed therapy on patients enrolled on phase II afatinib/paclitaxel or trastuzumab/pembrolizumab/chemotherapy trials(Janjigian/Lewis).- We successfully performed Zr-trastuzumab PET on 11 patients treated with anti- HER2 therapy (see figures and analysis below).

c. Analysis of HER2 expression on post treatment biopsies at week 2 on afatinib (Janjigian/ Vakiani) -We have analyzed HER2 expression on 8 post treatment biopsies at week 2 on afatinib.

Major Task 2-

d. Complete accrual to phase II trial of afatinib/paclitaxel in HER2+EG tumors (Janjigian) - 11-166 protocol accrual has closed. 8 patients have been treated total with Afatanib and Paclitaxel, 4 patients were treated since 9/2017.

e. Determine clinical efficacy of afatinib/paclitaxel in HER2+ EG tumors in Phase II setting (Janjigian/Lewis)- A total of 8 patients have been treated with Afatanib and Paclitaxel. The median age was 68.5. There were 5 males enrolled and 3 females. 4 patients are currently AWD, while 4 DOD. 3 patients experienced POD, 1 patient had SD, and 3 patients had a PR, while one patient has not yet been evaluated. Patients tolerated a median of 2.5 cycles [range: 1 – 7].

f. Collect tumor samples from patients enrolled on Phase II trial (Janjigian)- We successfully collected 88 samples of HER2 patients. We collected samples from 31 patients with HER2+ EG adenocarcinoma receiving trastuzumab/pembrolizumab and 8 patients treated with afatinib/paclitaxel.

g. Proteomic analysis of tumor samples (Lewis)—Completed on 3 patients

Specific Aim 2

Major Task 1-

a. Perform targeted exon sequencing on samples obtained from serial biopsies (Janjigian)- We successfully sequenced 37 HER2+ EG cancer obtained prior to initiation of trastuzumab based therapy. Additionally, we sequenced 34 samples from from patients on first-line pembrolizumab/ trastuzumab study, and were able to biopsy 10 patient at progression with 10 matched pre/post progression paired samples analyzed. Serial samples from the 8 patients on the phase II afatinib/paclitaxel trial has been sequenced with the analysis ongoing.

b. Perform Rapid Autopsies (Janjigian/Iacobuzio) —4 rapid autopsies were performed and data analysis is completed (see figures and analysis below).

c. Perform targeted exon sequencing, RNA-seq and miRNA-Seq profiling all matched primary tumor and all sites of metastasis obtained from rapid autopsies (Janjigian/ Iacobuzio)- This analysis is ongoing.

Major Task 2-

a. Bioinformatics and Biostatistician Analysis (Janjigian/Shultz/ Capanu)- Bioinformatics and Biostatistics have completed the analysis of related HER2 experiments and studies.

Specific Aim 3

Major task 1-

a. Establish Patient Derived Xenografts (PDXs) from biopsy samples (Janjigian/ De Stanchina)- We implanted 113 PDXs . We established 67 PDXs derived from 57 patients with HER2+

tumors. PDX models were also used to image response to treatment which has increased our understanding of resistance and will allow us to design future studies.

b. Histological review, molecular characterization and comparison of established PDXs to parental tumor to confirm accurate representation of the parental tumor (Janjigian/Vakiani)- Targeted sequencing was performed using MSK-IMPACT next-generation sequcing platform. The PDXs have corresponding clinical sequencing from patient tumors allowing genomic fidelity between patient and PDX samples. We have sequenced a total of 49 PDX samples. Of these, 20 were HER2+. There are another 23 PDXs currently pending sequencing.

Major Task 2-

a. Establishment of organoids from PDX samples (Lewis)- We have successfully collected samples for organoids for 8 HER2 patients.

Specific Aim 4

Major Task 1-

a.Therapeutic experiments on established PDXs to evaluate how the genomic alterations impact sensitivity to next generation of therapies (Janjigian/ de Stanchina/Tavazoie)- Therapeutic experiments were performed to determine the sensitivity of tumor GD to afatinib or pertuzumab + Taxol combination.

b.Perform gain/loss of function studies afatinib-sensitive *ERBB2*-amplified cell lines resistant to this agent (Janjigian/ de Stanchina/Tavazoie)- This was attempted but was not successful due to failure for cell lines to grow. We are currently studying this in organoids.

c.shRNA kinase library screen (Janjigian/ de Stanchina)- This was attempted but was not successful due to failure for cell lines to grow. We are currently studying this in organoids.

Major Task 2-

a.Progression passages of PDX models of HER2+ gastric cancer for *in vivo* selection of metastasis (Tavazoie)- Progression and passages of eight PDX models of HER2 gastric cancer for in vivo selection of metastasis.

Major Task 3-

a.microRNA profiling studies (Tavazoie)- micro RNA profiling of isogenic highly or lowly metastatic HER2 amplified PDX has been completed.

b.Analysis of microRNA profile results obtained (Tavazoie)- microRNA profile results have been obtained.

Major Task 4-

a.Advise in functional testing of candidate genes (Tavazoie)- This is ongoing.

Specific Aim 1

89Zr-trastuzumab PET

We successfully performed Zr-trastuzumab PET on 11 patients since September 2016.

We hypothesized that functional imaging with 89Zr-trastuzumab PET could identify afatinib-sensitive and afatinib-resistant tumor sites prior to evidence of clinical progression. These tumor sites could then be explored post-therapy. To explore this hypothesis, we performed 89Zr-trastuzumab PET imaging before treatment and before the first CT assessment (after 3–5 weeks on therapy) in 8 patients, one treated with afatinib monotherapy and seven with afatinib/trastuzumab. All patients demonstrated 89Zr-trastuzumab tumor uptake in at least one disease site prior to therapy initiation. In the pre-treatment scans, we observed a wide range in median standardized uptake values (SUV) among patients and among lesions in individual patients (median SUVmax 15.6; range, 6.4 to 23.8; Figure 3C). Four patients (50%) had visible metastases on CT that did not exhibit 89Zr-trastuzumab uptake on PET, indicating pre-treatment intra-patient HER2 expression heterogeneity between sites. All 4 of these patients demonstrated disease progression within 6 weeks. Even when mean 89Zr-trastuzumab uptake declined 3–5 weeks post-therapy, we observed intra-patient lesion-to-lesion variability in this change (Fig. 1C). Notably, the 3 patients with tumor regression on afatinib and afatinib/trastuzumab therapy had uniformly high 89Zr-trastuzumab uptake (SUV >5) in all lesions visualized on CT (see Fig. 3C). These data suggest that homogenous pretreatment 89Zr-trastuzumab uptake may be a marker of afatinib sensitivity and that heterogeneous uptake at baseline or variable 89Zr-trastuzumab PET response may be indicators of poor response to afatinib in ERBB2-amplified EG cancer.

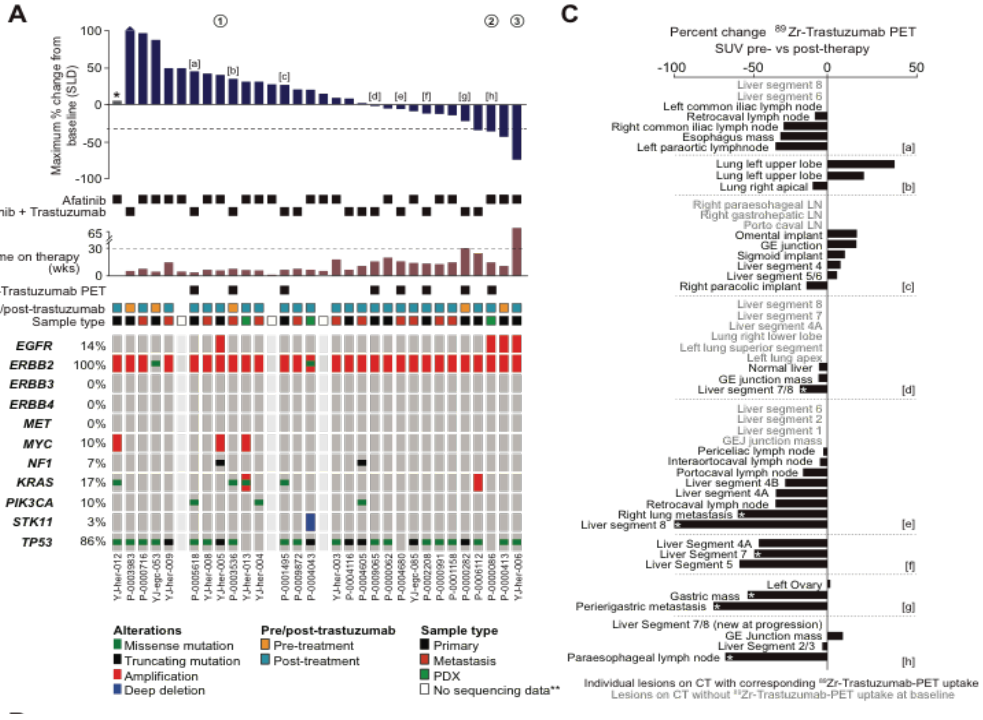

Figure 1: (A) Individual treatment outcomes. (C) Percent change 89Zr-trastuzumab SUV pre- and post-therapy.

Figure 1 shows individual treatment outcomes in 20 patients treated with afatinib and 12 patients treated with afatinib and trastuzumab. A percentage best change from baseline in the target lesion assessed by RECIST 1.1. Relevant clinical features (time on therapy, type of sequenced sample, and time point of the sequenced sample) plus key genomic alterations in sequenced samples are shown for each patient. SLD, sum of longest diameter on CT scan. *Non-evaluable. **Sample uninformative due to low tumor content. B, dual probe EGFR and ERBB2 FISH from tumor biopsy collected prior to protocol treatment demonstrated diffuse and uniform ERBB2 and EGFR co-amplification in virtually all tumor cells. There was no additional tissue on P-0000413. C, Percent change in 89Zr-trastuzumab PET standard uptake value (SUV) pre- vs post-therapy for each individual lesion on CT in 1 patient treated with afatinib monotherapy and 7 patients treated with afatinib/trastuzumab. White stars inside the bar plots denote lesions with resolution of uptake to baseline SUV.

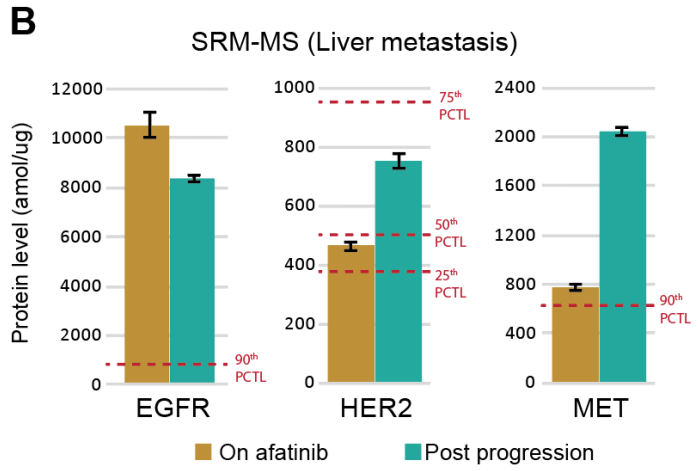

Figure 2: Proteomic analysis of HER2 samples from patient treated with afatinib therapy

As shown in figure 2, mass spectroscopy proteomic analysis of the tumor samples from patient 2 with HER2 amplified trastuzumab refractory gastric cancer and dramatic response demonstrated high level of EGFR expression on mass spectrometry. In this patient response was relative transient likely related to high level of MET expression seen in the tumor. The variation of EGFR, MET and HER2 amplification may shed light on the variation in response. This analysis is ongoing.

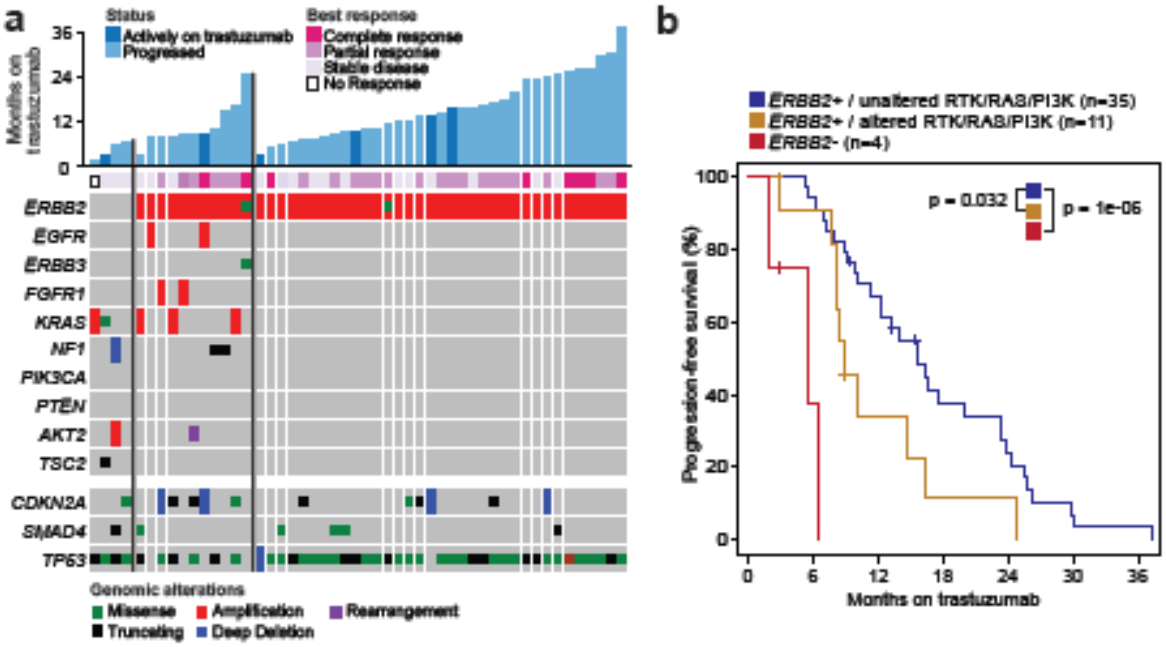

Figure 3: Genomic characterization of intrinsic and acquired trastuzumab resistance in patients with HER2+ esophagogastric cancer.

Figure 3 shows: (A) Duration and best response on first-line trastuzumab/chemotherapy for 50 patients with HER2-positive metastatic EG cancer is shown integrated with pre-treatment somatic genomics (legend, as indicated). (B) Kaplan-Meier progression free survival curves showing favorable outcome in ERBB2-amplified RTK/RAS/PI(3)K-wildtype tumors. Patients with *ERBB2*-negative by IMPACT or *ERBB2*+ and RTK/RAS/PI(3)K pathway activated tumors have significantly shorter time to progression on first-line trastuzumab therapy. (C) Analysis of somatic alterations in 23 pairs of matched pre- and post-trastuzumab samples. The oncoprint illustrates several oncogenic alterations that are shared between or private to the paired pre- and post- samples. (D) A representative case that illustrates loss of *ERBB2* in the post-treatment sample, which was validated by H&E, FISH, and IHC on the matched pre- and post-treatment specimens. (E) The anatomy of the acquired ERBB2 exon 16 deletion in the post-trastuzumab specimen.

Specific Aim 2

We successfully sequenced 37 HER2+ EG cancer obtained prior to initiation of trastuzumab based therapy. Additionally, we sequenced 34 samples from from patients on first-line pembrolizumab/ trastuzumab study, and were able to biopsy 10 patient at progression with 10 matched pre/post progression paired samples analyzed.

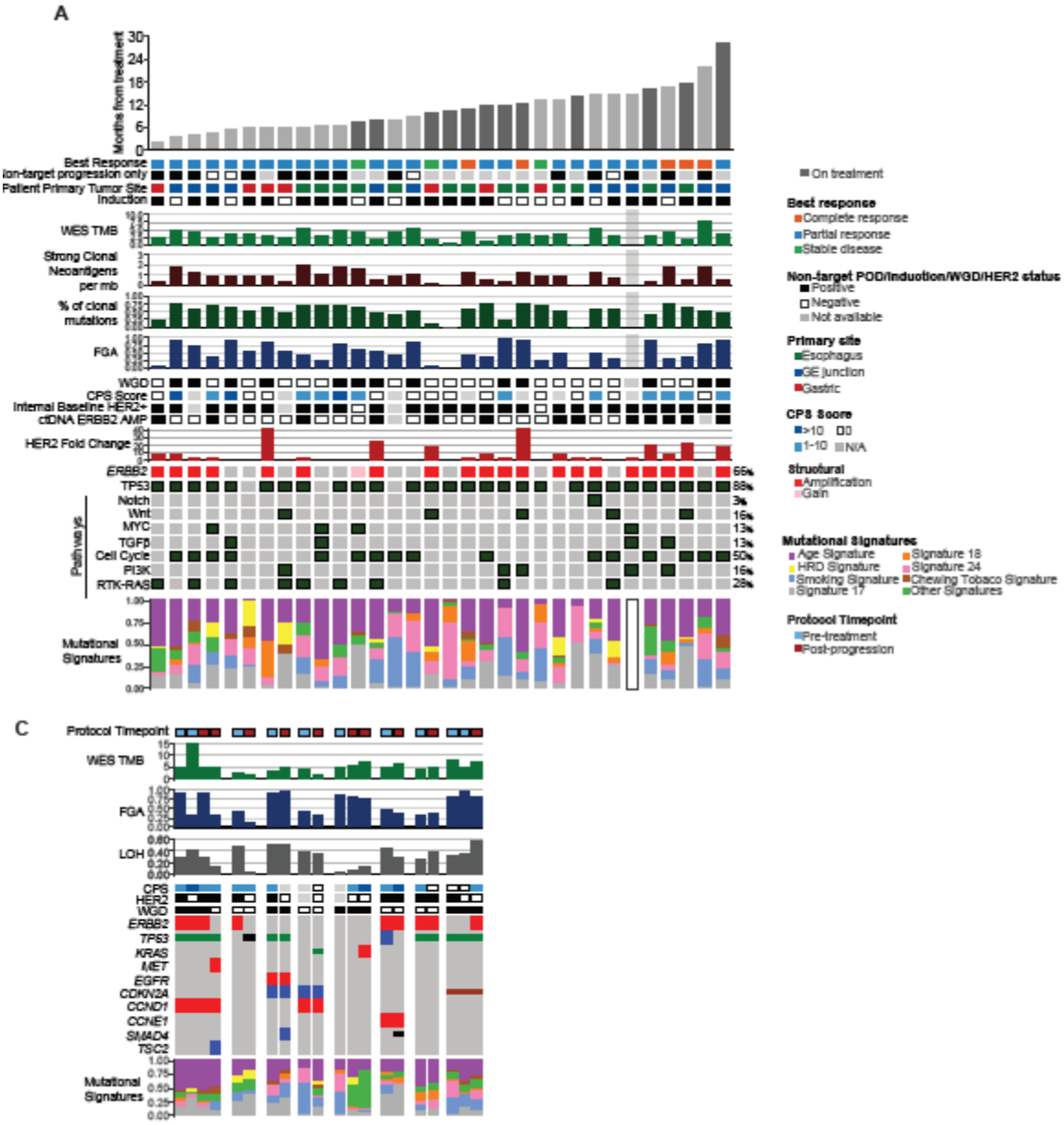

Figure 4: (a) co-occurring genomic alterations, tumor mutation burden, PDL-1, and MSK HER2 status (n=29), (c) Determinants of resistance to therapy in HER2 patients treated with trastuzumab and pembrolizumab

As shown in figure 4, of 37 HER2+ patients treated with trastuzumab in combination with pembrolizumab, 32 were sequenced using MSK-IMPACT and 31 were also analyzed by whole exome sequencing (WES). Figure 4A indicates co-occurring genomic alterations, tumor mutation burden, PDL-1

status, and HER2 status at MSK. In select patients who initially responded and later progressed on therapy, we performed research biopsies to allow for analysis to uncover determinants of resistance to therapy. Three of the 8 patients demonstrated new, activating alterations in the RTK/RAS pathway, including *KRAS* amplification, *KRAS* mutation, and *MET* amplification (Figure 4C). Two of the 8 patients (neither of whom developed an activating MAPK pathway alteration), demonstrated loss of *ERBB2* amplification at progression. Of the remaining fourthree patients, the post-progression tumors of two patients had inactivating alterations in *SMAD4*.

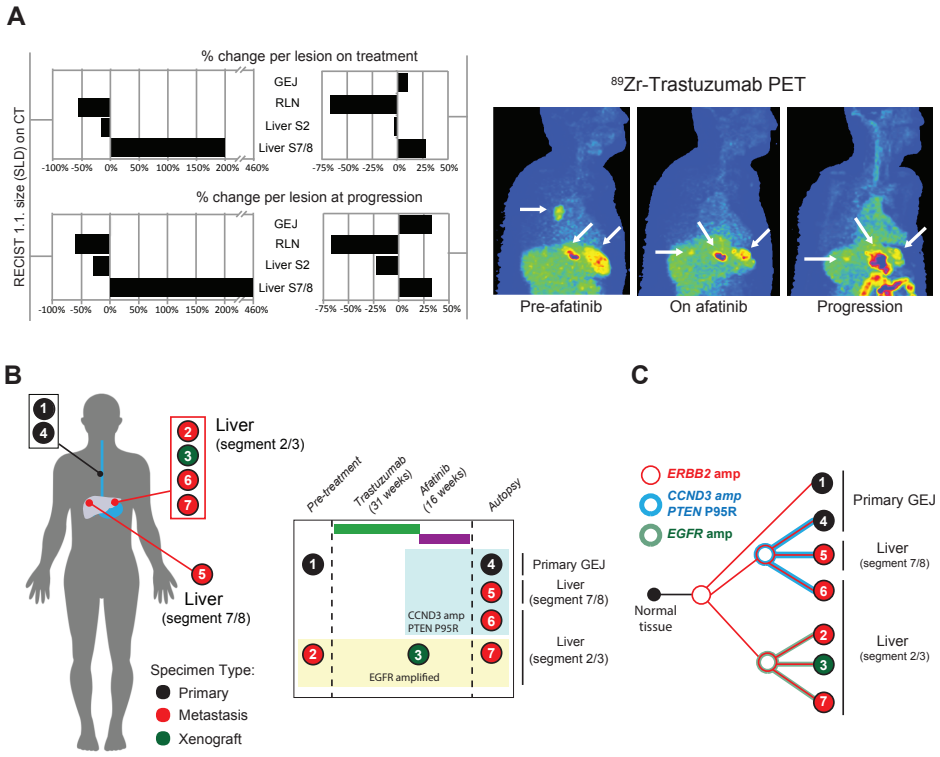

Figure 5: Intrapatient disease heterogeneity and lack of EGFR amplification as mechanism of afatinib resistance in patient P-0000086.

Figure 5 shows intrapatient disease heterogeneity and lack of EGFR amplification as mechanism of afatinib resistance in patient P-0000086. (a) Radiographic tumor assessment using 89Zr-trastuzumab PET and conventional CT scan, with corresponding bar graphs. 89Zr-trastuzumab PET values in SUV and CT measurements in mm. 89Zr-trastuzumab PET images include three panels: left panel (pre-afatinib) is a baseline pretreatment image showing uptake in segment 2/3 liver metastasis (SUV 16.4), GE junction (SUV 21.5) and retroesophageal lymph node (SUV 9.1); middle panel (on afatinib) corresponds to 3 weeks post-treatment initiation, showing resolution of retroesophageal lymph node (SUV 3.0) with decrease in size but persistent high uptake in segment 2/3 liver lesion (SUV 15.8), similar uptake in GE junction (SUV 23.7) and a new lesion in liver segment 7/8 appeared on 89Zr-trastuzumab PET (SUV 8.6); right panel (progression) shows post-progression 89Zr-trastuzumab PET uptake in GE junction (SUV 28.5) and segment 7/8 liver lesion (SUV 9.0), decreased uptake in liver segment 2/3 (SUV 12.3) and ongoing resolution of retroesophageal lymph node. (b) Genomic comparison of matched pre- and post-treatment primary and liver segment 2/3 and post-treatment progression liver segment 7/8 obtained at warm autopsy. EGFR amplification was unique to the segment 2/3 liver lesion with ongoing response. The enlarging GE junction tumor and segment 7/8 liver metastasis were not EGFR-amplified. In addition to a likely pathogenic mutation in PTEN (P95R, also present in a segment 2/3 liver lesion), the non-

responding metastases acquired an amplification of CCND3. SUV, standardized uptake value on 89Zr-trastuzumab PET; SLD, sum of longest diameter on CT scan. (c) Phylogenetic tree showing the inferred patterns of clonal evolution for the samples described in Figure 5b.

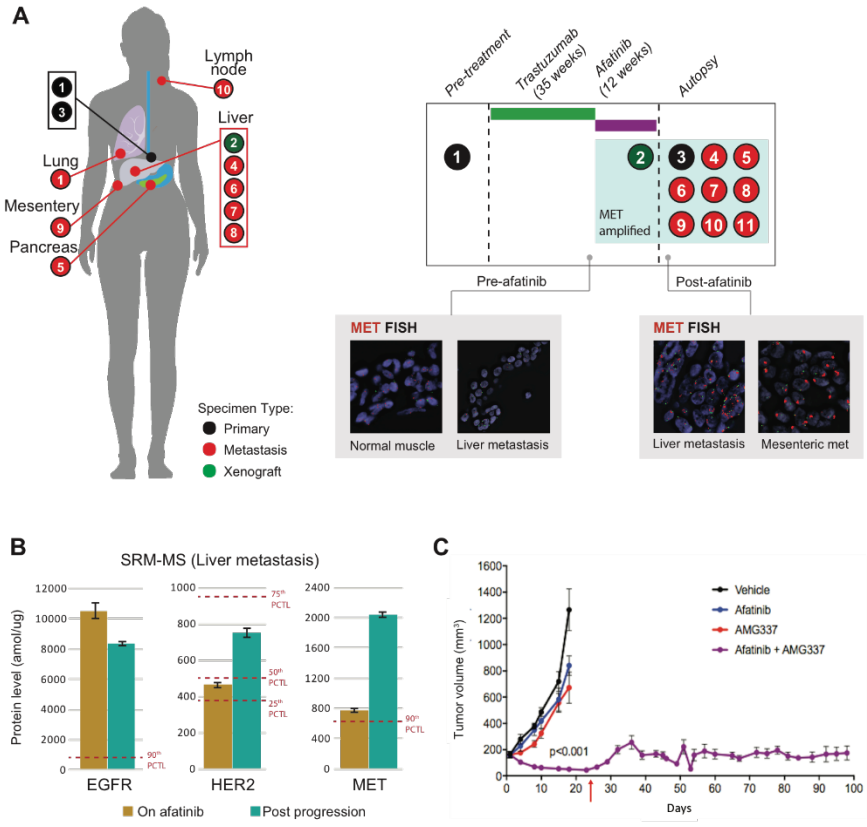

Figure 6: Acquired MET amplification as a mechanism of afatinib resistance in patient P-0000413 with EGFR- and ERBB2-amplified EG cancer

Figure 6 shows acquired MET amplification as a mechanism of afatinib resistance in patient P-0000413 with EGFR- and ERBB2-amplified EG cancer. (a) Genomic comparison of matched pretreatment biopsy with post-treatment metastases obtained at warm autopsy demonstrating acquired MET amplification. (b) SRM-MS analysis of liver metastasis sample obtained on afatinib with post-progression autopsy sample revealing increased MET and HER2 and decreased EGFR expression. (c) Efficacy of afatinib and afatinib/AMG 337 in MET/EGFR/ERBB2-amplified PDX model established from patient P-0000413. The combination of afatinib/AMG 337 resulted in complete tumor response at 21 days in MET/EGFR/ERBB2-amplified PDX. Red arrow indicates when the mice were rechallenged with the afatinib/AMG 337 combination following discontinuation of drug treatment, with durable tumor control again achieved.

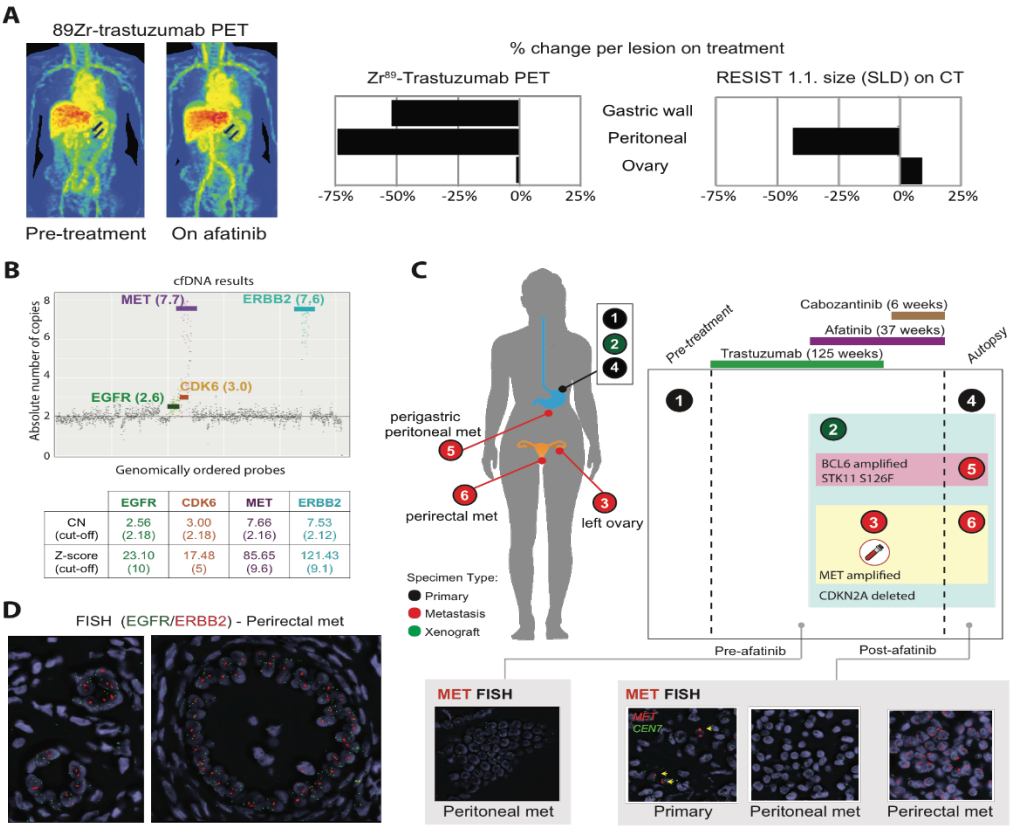

Figure 7: Acquired MET amplification as a mechanism of afatinib/trastuzumab resistance in patient P-0000282 with ERBB2-amplified EG cancer

Figure 7 shows acquired MET amplification as a mechanism of afatinib/trastuzumab resistance in patient P-0000282 with ERBB2-amplified EG cancer. (a) Radiographic tumor assessment using 89Zr-trastuzumab PET and conventional CT scan, with corresponding bar graphs. 89Zr-trastuzumab PET values in SUV and CT measurements in mm. 89Zr-trastuzumab PET, left panel, is a baseline pretreatment image showing uptake in left ovary metastasis (SUV 5.1) not visible in this projection, perigastric peritoneal metastasis (SUV 7.2, lower arrow) and gastric mass (SUV 8.2, upper arrow). Right panel is 89Zr-trastuzumab PET 5 weeks post-treatment initiation, showing resolution of uptake in perigastric peritoneal metastasis (SUV 1.8) and gastric mass (SUV 3.9) and persistently high uptake in left ovarian metastasis (SUV 5.2) not seen on MIP images. (b) cfDNA analysis at the time of disease progression on afatinib/trastuzumab, demonstrating co-occurring ERBB2 and MET amplifications. (c) Genomic comparison of matched pretreatment biopsy with post-treatment metastases obtained from left ovarian metastasis and subsequently at rapid autopsy, demonstrating acquired MET amplification unique to the progressing lesions. The perigastric metastasis and the primary tumor, both of which demonstrated ongoing response to afatinib/trastuzumab, did not harbor MET amplification. (d) Dual probe EGFR and ERBB2 FISH from perirectal tumor biopsy collected after treatment demonstrated ERBB2 amplification and low-level gain of EGFR in a subset of tumor cells.

Specific Aim 3

Patient Derived Xenografts (PDXs)

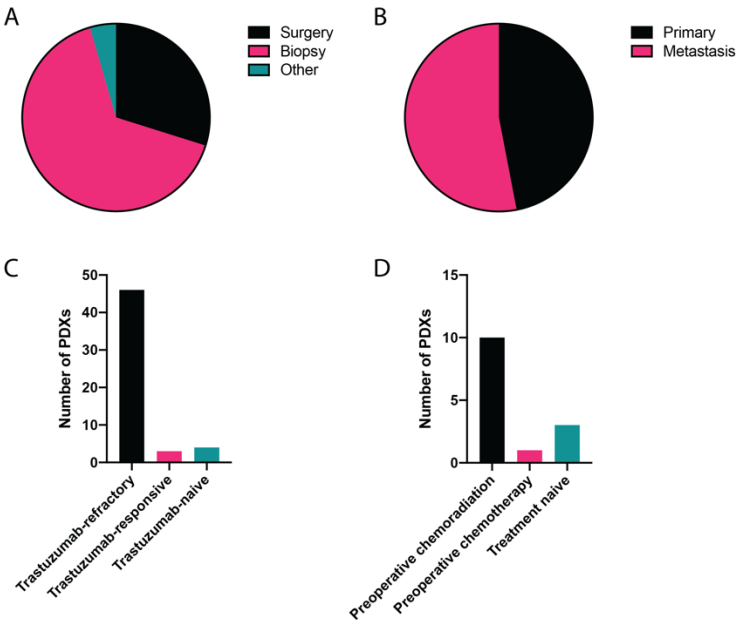

Figure 8. Overview of HER2-positive esophagogastric cancer PDXs. (a) Procedure for generation of PDXs. (b) Tissue origin (primary tumor or metastasis) for PDXs. (c) Treatment history with trastuzumab for HER2-positive PDXs derived from patients with metastatic disease. (d) Preoperative treatment history for PDXs derived from patients with localized HER2-positive tumors.

Table 1. Patient and PDX characteristics

Characteristic	N (%)
Gender (%)	
Women	11 (19)
Men	46 (81)
Anatomic tumor location (%)	
Gastroesophageal junction	18 (31)
Esophagus	18 (31)
Stomach	22 (38)
Stage at initial PDX collection (%)	
IV	43 (75)
III	8 (14)
II	5 (9)
I	1 (2)
Number of PDX per patient (%)	
1	46 (81)
2	9 (16)
3	1 (2)
Sample Type (%)	
Primary	31 (47)
Metastasis	33 (53)
Procedure Type	
Surgery	20 (30)
Biopsy	44 (66)
Other	3 (4)

As shown in figure 8 and table 1, we have generated a collection of 113 patient-derived xenograft (PDX) models from patients with gastric, esophageal and gastroesophageal junction cancers. Of these 113 PDXs, 67 PDXs were derived from 57 patients with HER2-positive tumors, including patients with metastatic (43 patients, 75%) and early stage/locally advanced (14 patients, 25%) cancers at the time of PDX collection. The PDXs were generated from either surgical resections or biopsies (Figure 8a) using tissue from the primary esophagogastric tumor or a metastatic site (Figure 8b). In addition, for some HER2-positive patients, we have derived two (9 patients) or three (1 patient) PDXs from the same patient at different timepoints within the treatment course. The large majority of PDXs from patients with metastatic HER2-positive cancers were generated from patients with treatment-refractory disease with progression on trastuzumab and chemotherapy containing regimens (Figure 8c). Other PDXs were derived from patients with newly diagnosed metastatic disease or with ongoing responses to trastuzumab-containing therapy. Moreover, we have generated several HER2-positive esophagogastric cancer PDXs from patients with localized disease, including PDXs from untreated tumors and tumors exposed to preoperative chemotherapy or chemoradiation (Figure 8d).

We have performed targeted sequencing of most of the HER2-positive PDXs using the MSK-IMPACT next-generation sequencing platform, with the latest version including 468 genes. The majority of these PDXs have corresponding clinical sequencing from patient tumors, allowing for analysis of genomic fidelity between patient and PDX samples. Frequently co-occurring alterations with HER2 amplifications include *TP53* mutations, cell-cycle gene alterations (i.e. *CDK12*, *CCDN3, CDKN2B*, *CCNE1*) and receptor tyrosine kinase alterations (such as *EGFR*), consistent with patient sequencing data.

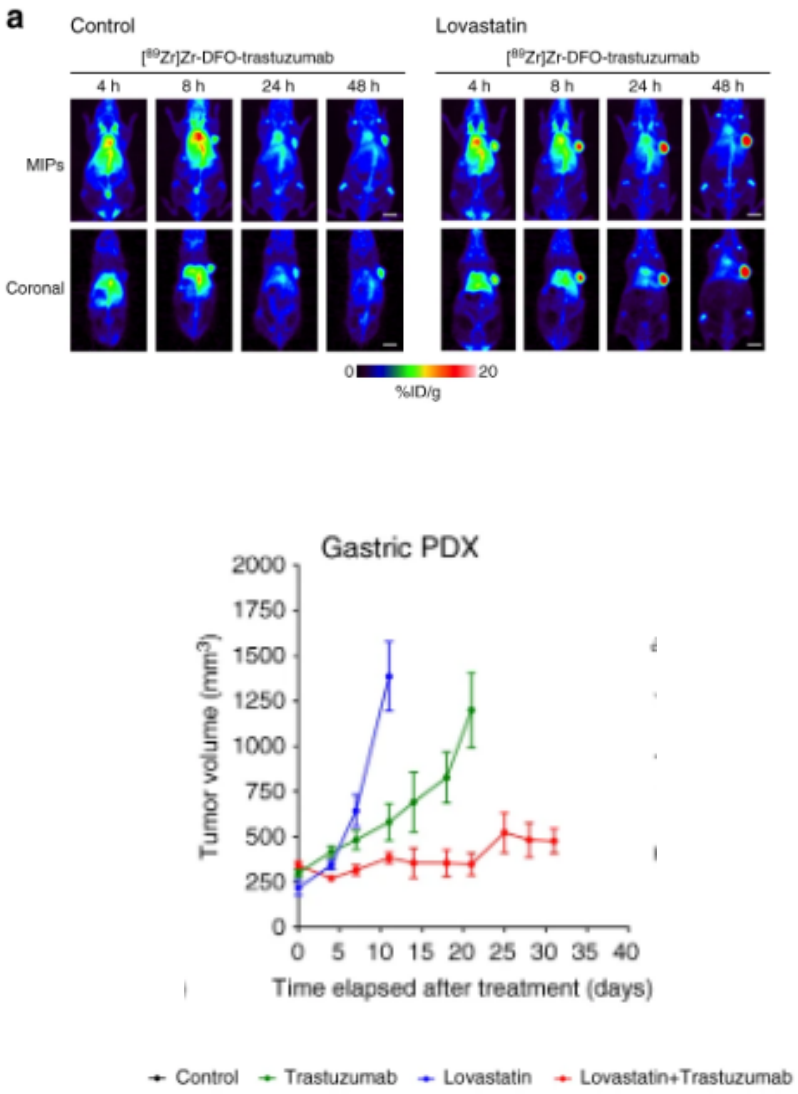

Figure 9: (a) Use of PDXs to image response to treatment. (b) Superior in vivo therapeutic efficacy of trastuzumab combined with lovastatin when compared with trastuzumab in nu/nu NSG mice bearing gastric PDXs (n≥8 mice per group).

As shown in figure 9, statin treatment increases tumor uptake and efficacy of trastuzumab. The use of gastric PDXs to image response to treatment and the therapeutic efficacy of trastuzumab combined with lovastatin increases our understanding of mechanisms of resistance and will assist in designing future studies.

Specific Aim 4

Generate highly liver metastatic PDX with in-vivo selection and therapy

Progression and passages of eight PDX models of HER2 gastric cancer for in vivo selection of metastasis.

Table 2:PDXs generated for *in vivo* liver selection

PDX ID	Clinical Key	Derivative
GAS CX	T3N1 GEJ adenocarcinoma	LVM1a
GAS DF1	T1N1M1 Gastric adenocarcinoma	LVM1a, b, and LVM2

Where, LVM1 stands for liver metastatic 1st passage line and LVM2 stands for liver metastatic 2nd passage line.

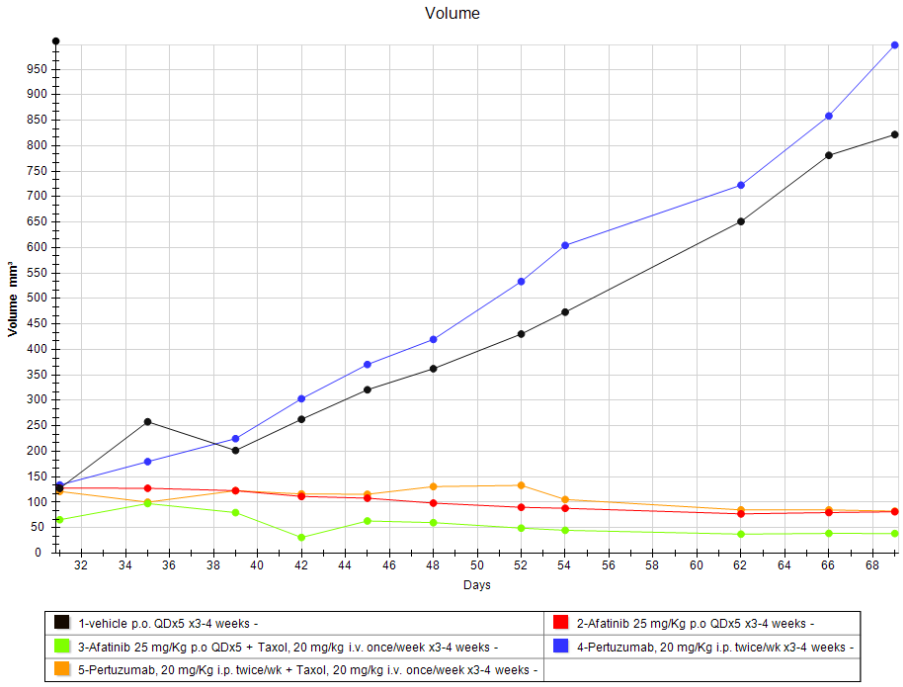

Figure 10: **GD PDX**: Determined the sensitivity of tumor GD to afatinib or pertuzumab + Taxol combination.

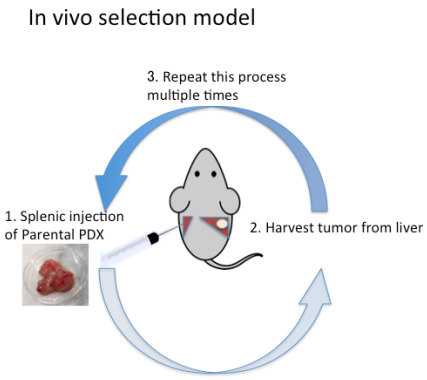

Figure 11: Schema of in-vivo selection model

Figure 11 shows single cell solution from parental PDXs are injected to spleen. Injected PDXs will colonize in liver through portal circulation. Harvest the colonized metastatic tumor and repeat the same process.

Figure 12:Ex-vivo image of highly liver metastatic derivative (LVM=upper and Parental=lower).

Table 3. List of highly liver metastatic PDX derivative

PDX ID	Clinical Key	Derivative
GAS DK	T3N0 Gastric Tubular adenocarcinoma	LVM1b
GAS DK	T3N0 Gastric Tubular adenocarcinoma	LVM1a
GAS CX	T3N1M1 GEJ cancer	LVM1a
GAS DQ	T1N0 Signet Ring cell Gastric cancer	LVM1a
GAS DC	Stage IV T3N1 GEJ Adenocarcinoma	LVM1a
GAS EK	Stage IV TXN0M1 GEJ adenocarcinoma	LVM1a
GAS DO	Stage IV T3N3M0 Gastric Tubular Adenocarcinoma	LVM2a
GAS DS	Stage IV T1N0M1 Gastric Signet Ring Cell Adenocarcinoma	LVM1a

micro RNA profiling of isogenic highly- or lowly- metastatic HER2 amplified gastric PDX

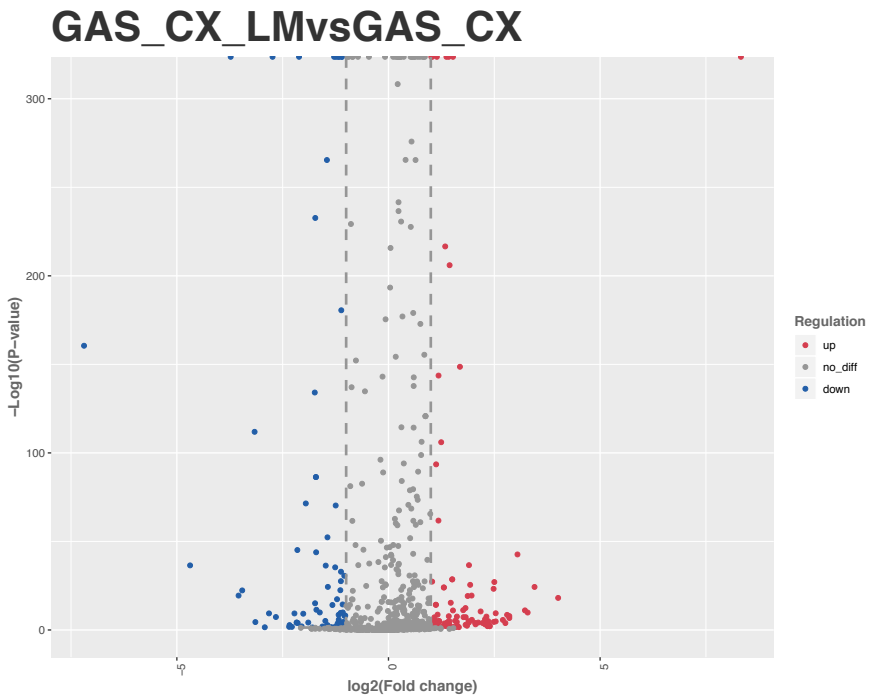

Figure 13: Volcano plot of up- or down- regulated micro RNAs

Sample: Gastric CX PDX, T3N1M0, tissue was taken from the stomach, HER2 amplified

Results: Most highly down regulated miRs with log2FC > 3 are annotated.

Table 2. List of top 20 differentially expressed miRs

Index	miR_name	miR_seq	up/down	fold_char	log2(fold	pvalue(ch	pvalue(fi
1	hsa-miR-1-3p	TGGAATGTAAAGAAGTATGTAT	down	0.01	-7.19	0.00E+00	########
2	hsa-miR-155-5p_R-1	TTAATGCTAATCGTGATAGGGGT	down	0.04	-4.69	5.06E-29	3.24E-37
3	hsa-miR-363-3p	AATTGCACGGTATCCATCTGTA	down	0.08	-3.73	0.00E+00	0.00E+00
4	hsa-miR-1911-5p	TGAGTACCGCCATGTCTGTTGGG	down	0.09	-3.54	3.48E-17	3.70E-20
5	hsa-miR-218-5p_R+2	TTGTGCTTGATCTAACCATGTGA	down	0.09	-3.46	7.96E-20	4.46E-23
6	hsa-miR-20b-5p	CAAAGTGCTCATAGTGCAGGTAG	down	0.11	-3.16	0.00E+00	########
7	bta-miR-1911_R+1	TGAGTACCGCCATGTCTGTTGGGA	down	0.11	-3.14	5.74E-05	3.37E-05
8	hsa-miR-548i	AAAAGTAATTGCGGATTTTGCC	down	0.13	-2.92	3.38E-02	2.90E-02
9	hsa-miR-1269b	CTGGACTGAGCCATGCTACTGG	down	0.14	-2.82	3.81E-09	4.21E-10
10	hsa-miR-486-5p	TCCTGTACTGAGCTGCCCCGAG	down	0.15	-2.74	0.00E+00	0.00E+00
11	hsa-miR-584-5p_R-1	TTATGGTTTGCCTGGGACTGA	down	0.16	-2.66	2.03E-07	4.72E-08
12	hsa-miR-6511b-3p_R-1	CCTCACCACCCCTTCTGCCTGC	down	0.20	-2.35	1.02E-02	1.72E-02
13	mmu-miR-3968_L-2_1ss14AT	AATCCCACTCCTGACACCA	down	0.20	-2.34	2.50E-03	1.72E-03
14	hsa-miR-4517_R+1	AAATATGATGAAACTCACAGCTGAGG	down	0.21	-2.28	1.37E-02	1.72E-02
15	mdo-miR-215_R+1_1ss20AC	ATGACCTATGAATTGACAGCCAT	down	0.21	-2.22	2.08E-09	4.49E-10
16	mmu-mir-3535-p3	AGATAGTCAAGTTCTGATC	down	0.22	-2.17	7.60E-05	6.56E-05
17	mmu-mir-3535-p5	AGATAGTCAAGTTCTGATC	down	0.22	-2.17	7.60E-05	6.56E-05
18	hsa-miR-181a-2-3p	ACCACTGACCGTTGACTGTACC	down	0.22	-2.15	7.18E-42	7.02E-46
19	PC-3p-43750_38	TATATATAGTATATGTGCATGT	down	0.23	-2.14	2.91E-04	1.10E-04
20	hsa-miR-10b-5p_R-1	TACCCTGTAGAACCGAATTTGT	down	0.23	-2.11	0.00E+00	0.00E+00

Known functions of top 5 down-regulated miRs in cancers

- miR-1-3p is known to regulate bladder cancer proliferation by up-regulating SFRP1 expression and is also known for inhibiting c-Met in NSCLC.
- miR-155 is known to be down regulated in FAP patients and modulate WNT signaling by targeting AXIN1 and TCF4.
- miR-363-3p is known to inhibit EMT and metastasis in CRC by targeting Sox4.
- miR-1911-5p is associated with metastasis phenotype in CRC.
- miR-218-5p_R+2 is known to inhibit cancer cell proliferation and migration via EGFR in NSCLC.

To identify a key gene in promoting gastric cancer liver metastasis, we generated an isogenic highly liver metastatic derivative from patient derived xenografts of gastric cancers (Gas PDXs) by *in vivo* selection. (Fidler, 1973) Iterative repeats of parental Gas PDXs injection to the portal circulation enriched highly liver metastatic Gas PDX sub-populations (Gas LVM PDXs). Over a decade, many groups including us (Loo et al., 2015; Ma et al., 2007; Pencheva et al., 2012; Tavazoie et al., 2008) have shown that differentially expressed micro RNAs regulate cancer metastasis. However, the role of differentially expressed micro RNAs in gastric cancer metastasis is little known, which motivated us to conduct micro RNA profiling of Gas LVM PDX and isogenic parental Gas PDX. We conducted micro RNA profiling of HER2 amplified Gas PDX, Gas CX and its LVM derivative, Gas CX LVM revealing 24 significantly differentially expressed micro RNAs with the cut off of log2FC=2 and p-value<0.05. Among the 24 highly significant micro RNAs, miR-1-3p is known to regulate bladder cancer proliferation by up-regulating sFRP1 expression(Shang et al., 2017). sFRP1 is known to promote gastric cancer proliferation by inhibiting TGF beta/Smad3 signaling(Peng et al., 2019). Taken together, miR-1-3p down-regulation we saw in our analysis might have an important regulatory role in gastric cancer metastasis although we must experimentally validate the hypothesis. In summary, micro RNA profiling of LVM and parental Gas PDX revealed novel differentially expressed micro RNAs and the experimental validation of those findings and further mechanistic investigation is highly warranted.

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Peng, J.X., Liang, S.Y., and Li, L. (2019). sFRP1 exerts effects on gastric cancer cells through GSK3beta/Rac1mediated restraint of TGFbeta/Smad3 signaling. Oncol Rep *41*, 224-234.
Shang, A., Yang, M., Shen, F., Wang, J., Wei, J., Wang, W., Lu, W., Wang, C., and Wang, C. (2017). MiR-1-3p Suppresses the Proliferation, Invasion and Migration of Bladder Cancer Cells by Up-Regulating SFRP1 Expression. Cell Physiol Biochem *41*, 1179-1188.
Tavazoie, S.F., Alarcon, C., Oskarsson, T., Padua, D., Wang, Q., Bos, P.D., Gerald, W.L., and Massague, J. (2008). Endogenous human microRNAs that suppress breast cancer metastasis. Nature *451*, 147-152.

IMPACT

What opportunities for training and professional development has the project provided?

Reflecting her academic and research achievements, in 2018, Dr. Janjigian was appointed Chief of MSK's Gastrointestinal Oncology Service. This service is one of the largest at MSKCC and Dr. Janjigian's selection as its leader after an international search is a testament to her remarkable accomplishments.

How were the results disseminated to communities of interest?

National meetings and manuscripts published in Cancer Discovery-

Cancer Discov. 2018 Jan;8(1):49-58. doi: 10.1158/2159-8290.CD-17-0787. Epub 2017 Nov 9. PMID: 29122777

Cancer Discov. 2019 Feb;9(2):199-209. doi: 10.1158/2159-8290.CD-18-0598. Epub 2018 Nov 21.PMID:30463996.

What do you plan to do during the next reporting period to accomplish the goals?

N/A

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

CHANGES/PROBLEMS

Changes in approach and reasons for change

N/A

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

PRODUCTS

- **Publications, conference papers, and presentations**

Janjigian et al EMSO 2016 conference Annals of Oncology (2016) 27 (6): 207-242. 10.1093/annonc/mdw371

Janjigian Y, et al. Genetic predictors of response to systemic therapy in esophagogastric cancer. *Cancer Discov.* 2018 Jan;8(1):49-58.

Sanchez-Vega F, et al.EGFR and MET Amplifications Determine Response to HER2 Inhibition in ERBB2-Amplified Esophagogastric Cancer. *Cancer Discov.* 2019 February.

Pereira PMR, Sharma SK, Carter LM, Edwards KJ, Pourat J, Ragupathi A, **Janjigian YY**, Durack JC, Lewis JS. Caveolin-1 mediates cellular distribution of HER2 and affects trastuzumab binding and therapeutic efficacy. *Nat Commun*. 2018 December 3. PMID:30510281.

O'Donoghue JA, Lewis JS, Pandit-Taskar N, Fleming SE, Schoder H, Larson SM, Beylergil V1, Ruan S, Lyashchenko S, Zanzonico PB, Weber WA, Carrasquillo JA, **Janjigian YY**. Pharmacokinetics, biodistribution, and radiation dosimetry for 89Zr-trastuzumab in patients with esophagogastric cancer. *J Nucl Med*. 2017 Jun 21 PMID: 28637800.

- **Books or other non-periodical, one-time publications.**

Nothing to report

- **Other publications, conference papers, and presentations**

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Improved efficacy of tumor implantation to immune deficient mice

1. Dissect human tumor to 100mm3 pieces
2. With blunt edge of scissors, scrape off soft/whitish necrotic tissue
3. Cut the fragment to 20-30mm3 pieces by removing non-viable tissue
4. Implant these 20-30mm3 pieces in bilateral flank of immune deficient mice as described anywhere.

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Personnel	Role	Percent effort
Yelena Janjigian	PD/PI	0.6
Christine Iacobuzio-Donahue	Co-PI	0.36
Francisco Sanchez-Vega	Post-Doctoral Associate	2.4
Hui-Yong Zhao	Laboratory Assistant	1.44
Brittanie Millang	Research Assistant	0.9

Has there been a change in the active other support of the PD/PI(s) or senior/key

personnel since the last reporting period?
Nothing to report.

What other organizations were involved as partners?
Nothing to report.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: ANNUAL REPORT SUBMITTED ON BEHALF OF DRS. LEWIS AND TAVAZOIE BY THE LEAD PI—DR. JANJIGIAN

APPENDICES
Nothing to report