Science Signaling
AAAS

www.sciencesignaling.org/cgi/content/full/11/551/eaat9773/DC1

Supplementary Materials for

Neratinib is effective in breast tumors bearing both amplification and mutation of ERBB2 (HER2)

Emiliano Cocco, F. Javier Carmona, Pedram Razavi, Helen H. Won, Yanyan Cai, Valentina Rossi, Carmen Chan, James Cownie, Joanne Soong, Eneda Toska, Sophie G. Shifman, Ivana Sarotto, Peter Savas, Michael J. Wick, Kyriakos P. Papadopoulos, Alyssa Moriarty, Richard E. Cutler Jr., Francesca Avogadri-Connors, Alshad S. Lalani, Richard P. Bryce, Sarat Chandarlapaty, David M. Hyman, David B. Solit, Valentina Boni, Sherene Loi, José Baselga, Michael F. Berger, Filippo Montemurro*, Maurizio Scaltriti*

*Corresponding author. Email: filippo.montemurro@ircc.it (F.M.); scaltrim@mskcc.org (M.S.)

Published 9 October 2018, *Sci. Signal.* **11**, eaat9773 (2018)
DOI: 10.1126/scisignal.aat9773

This PDF file includes:

Text S1. Clinical case details.

Patient 1: On Dec 1996, a breast cancer patient admitted at the Candiolo Cancer Institute-FPO underwent a radical mastectomy with dissection of a 19 mm, grade 2 infiltrating ductal carcinoma. Axillary lymph-node status was positive, with 12 metastatic out of 20 removed lymph-nodes. Immunostaining for the estrogen receptor (ER) was positive in 5% of tumor cells whereas staining for progesterone receptor (PR) was present in 40% of tumor cells. HER2 receptor immunostaining was positive in 95% of cancer cells.
The patient underwent adjuvant chemotherapy (six cycles of 5-fluorouracil (600 mg/m^2, epi-doxorubicin 75 mg/m^2 and cyclophosphamide 600 mg/m^2 given every three weeks) and her subsequent follow-up was unremarkable until January 2003, when she presented with a left-axillary adenopathy. Axillary dissection revealed presence of HER2-positive metastatic breast cancer in 16 out of 24 nodes. The patient received a second adjuvant treatment with docetaxel 100 mg/m^2 every three week and she responded until July 2006, when she progressed in the left breast with HER2-equivocal breast cancer. The tumor was irradiated and the patient was put on endocrine therapy with letrozole 2.5 mg/day. On June 2007, a new skin lesion on the left breast confirmed the appearance of a HER2-positive breast cancer metastasis. Trastuzumab-based therapy was started and maintained until May 2010, with the addition of cyclophosphamide and methotrexate after 13 months because the appearance of a new intra-abdominal lymph-nodes with significant FDG uptake. After 4 months of treatment, further progression of abdominal lymph-nodes was documented. In Sept 2009, she received 6 cycles of paclitaxel achieving a partial remission. From Feb 2010, she was put on maintenance treatment with trastuzumab and on May 2010 a CT-PET scan revealed further skeletal and abdominal lymph-node progression. The patient was then put on treatment with capecitabine in combination with lapatinib and, after three months of treatment, she progressed in the pelvis. A biopsy of the lesion confirmed the presence of HER2-positive breast cancer. The patient then received palliative care and died from the disease on June 2011.
Targeted exome sequencing using the IMPACT platform (see Methods) was performed on the first loco-regional recurrence biopsied in 2003 (pre-treatment #1), on a second biopsy obtained before trastuzumab administration on 2007 (pre-treatment biopsy #2) and on the biopsy obtained on 2010 after the patient progressed to anti-HER2 therapy (post-treatment biopsy). Importantly, we confirmed an *ERBB2* gene copy number gain in all three samples whereas the HER2 L755S mutation was present only in the last sample collected in 2010.

Patient 2: In June 2006, a 65-year-old woman admitted at the Hospital Universitario Madrid underwent left cervical lymphadenectomy that showed high grade TTF1-, EMA+, CEA+ carcinoma of unknown origin. After receiving adjuvant radiation therapy on that area, the patient presented a relapse in June 2007 and a new biopsy confirmed its potential breast origin. In August 2007, chemotherapy was started (cisplatin plus docetaxel), without remission. In Dec 2007, she underwent a new surgical resection of skin relapse, which resulted to be incomplete and stained positive for HER2 by IHC. In Feb 2008, a new line of chemo with paclitaxel plus trastuzumab was started (three cycles), with maintenance single-agent trastuzumab, achieving a complete remission of her disease. In Feb 2009, progressive disease to skin and submaxilar lymph node was observed. A new line of trastuzumab plus docetaxel was initiated, achieving again a complete remission. However, in Sept 2009, the patient experienced progressive disease in the same areas, and was treated with trastuzumab and vinorelbine, with no meaningful response. In March 2010, a new line of capecitabine plus lapatinib was also started, and, upon new skin progression, changed to herceptin plus lapatinib (May 2010). Due to intrinsic resistance to this new regimen, the patient started avastin plus erlotinib in July 2010, and, in Sept 2010, erlotinin was substituted with capecitabine and cyclophosphamide.
In Dec 2010, a new skin biopsy was obtained and the molecular profiling on this sample (OncoCarta Assay panel v1.0) did not show any actionable mutation. From Feb 2011 to Aug 2011

the patient was treated with a new line of gemcitabine and trastuzumab (6 cycles), followed by trastuzumab as maintenance therapy. In Sep 2011, she started eribulin and completed 4 cycles. In March 2012, because of a new local progression, she started nab-paclitaxel with good tolerance and response of the skin metastasis. The patient progressed in Nov 12 and a new biopsy showed HER2-positive (Ratio HER2/CEP 3.52), hormone receptors-negative breast carcinoma. Molecular profiling [by the Oncomine Focus Assay™, a multi-biomarker next-generation sequencing (NGS) platform for solid tumors] of this sample revealed a new *ERBB2* mutation D769Y.
In Jan 2013, she started trastuzumab plus T-DM1 with a very good response and almost complete clinically remission of her skin metastases. She completed 18 cycles of treatment, which ended in March 2014 because of a new lymph nodal progression. Then, she received 3 cycles of pertuzumab and trastuzumab before a new skin progression. After radiation therapy of the skin lesions, the patient started epirubicin in October 2014. In Feb 2015, because of new subcutaneous progression, she underwent 16 cycles of CMF. A new locoregional progression in May 2015 revealed an intravascular metastasis with vessel and lymphatic infiltration. She was then enrolled in a clinical trial testing an anti HER2/HER3 antibody sponsored by Merus, of which she received 4 cycles and then, because of a new progression, she started neratinib as compassionate use. She received neratinib (240 mg/daily) C1D1 starting in October 2016, achieving stable disease (SD) by RECIST 1.1 and clinical benefit with improvement of her skin metastasis. She completed 6 cycles until the disease progressed in March 2017.

Patient 3: A 52 years old woman admitted at the Peter MacCallum Cancer Centre was diagnosed with locally advanced inoperable breast cancer in late 2009. She received 4 cycles of AC followed by 6 cycles of TCH after which she underwent right mastectomy and axillary dissection (2/2010). The patient achieved near pCR in her breast, with intravascular micropapillary carcinoma remaining in the residual disease at surgery. Given that 10 out of 22 lymph nodes were involved, the patient underwent adjuvant chest wall and SCF irradiation before starting with one year of adjuvant trastuzumab. The patient experienced skin recurrence in July 2011 and was treated with trastuzumab monotherapy for 5 cycles with no benefit. The next line of therapy was docetaxel in combination with trastuzumab late 2011 until Sept 2012, with benefit. At disease progression, she started liposomal doxorubicin bi-weekly with trastuzumab and changed to capecitabine in Dec 2012. In March 2013, they detected a single cerebellar lesion, which was resected. The patient started trastuzumab nonotherapy until Aug 2013 when single agent carboplatin was added. She was then treated with capecitabine and lapatinib from Oct 2013 to May 2014, when she started T-DM1. She progressed in the skin after four cycles and started nab-paclitaxel in combination with trastuzumab and everolimus. At this stage, an *ERBB2* L313I mutation was found in her circulating tumor DNA (allele frequency 8.6%). Everolimus was not tolerated and both nab-paclitaxel and trastuzumab were ceased in Oct 2015 due to skin progression. She was then treated with neratinib (240 mg daily) over 9 cycles from Dec 2015 until Oct 2016.

Patient 4: A 44-year-old woman was diagnosed with de novo metastatic (lung, liver and bone) HER2-positive hormone receptor negative breast cancer in Sept 2011. She completed 8 cycles of docetaxel in combination with trastuzumab with durable CR in the liver. In April 2012, she underwent right mastectomy/axillary dissection. In Jan 2014, due to liver progression, she started a 2nd line therapy of therapy with vinorelbine plus trastuzumab with reduction in splenomegaly and improvement in lymphadenopathy and liver lesion. In Sept 2014, she recurred with splenomegaly and new lung nodules. She then re-commenced vinorelbine with trastuzumab until May 2015, when she progressed and started T-DM1. After a mixed response,
T-DM1 was ceased in Dec 2015 due to progressive disease. The patient then declined any systemic therapy for several months. In April 2016, her tumor was screened on the in-house NGS screening platform SEGMENT that confirmed the HER2-postive disease and found an *ERBB2* R456C mutation. In May 2016, she started oral capecitabine plus trastuzumab and in Jan 2017 she

switched to gemcitabine plus trastuzumab. In May 2017, the patient started the therapy with neratinib (160 mg/day) and went on for 6 months.

Patient A: In 1990 a female breast cancer patient was initially diagnosed with ductal carcinoma and underwent a lumpectomy. At the time of diagnosis, the patient was N0/M0 and the first presentation with metastasis was in Nov 2011. NGS was performed with Foundation One (a commercially available platform for capture-based exome sequencing) in 2013 and the patient was identified as having an *ERBB2* L755S mutation.
At the time of enrolment onto the SUMMIT study (Dec 2014) the 75-year-old patient was T4/N0/M1 and had skin metastasis. The patient was positive for the ER and negative for PR. A sample was removed from the left breast mass (primary tumor location) and the IHC score was 2+, while the FISH score was 2.10 (Pathvysion HER2 DNA Probe kit- Abbott Molecular Inc.). In the metastatic setting the patient was treated with paclitaxel, abraxane and aromasin. On the SUMMIT study, the patient received and was maintained on 240 mg/day neratinib monotherapy and was on treatment for 141 days.

Patient B: In 2011 a male breast cancer patient was diagnosed with metastatic ductal grade 4 un-differentiated carcinoma. NGS was performed by MSK-IMPACT and a HER2 A775_G776insYVMA mutation was identified. At the time of diagnosis, the patient was T4/N1/M1. At the time of enrolment into the SUMMIT study (July 2014) the 61-year-old male patient was T4/N1/M1 and had visceral metastasis in the lung, brain, heart and kidney as well as skin metastasis. The patient was ER positive and PR negative and the HER2 copy number was 6.30 using the Dako FISH PharmDx kit and had a 2.30 reading on the HER2/CEP17 ratio. From Nov 2011 to Apr 2014 the patient had prior therapy with paclitaxel/bevacizumab, tamoxifen, gemcitabine/carboplatin and vinorelbine. In Apr 2014, the patient had palliative radiation in his left chest wall and in June 2014 the patient had palliative radiation in his brain. On the SUMMIT study, the patient received and was maintained on 240 mg/day neratinib monotherapy and was on treatment for 122 days.

Patient C: In June 2011 a female breast cancer patient was diagnosed with grade 3, poorly differentiated ductal carcinoma (T1/N1/M0). In July of 2011 the patient had a left total mastectomy and axillary node dissection and had an *ERBB2* FISH score of 1.40 (Vysis FISH Probe Kit- Abbott Molecular) and was ER/PR positive. In the adjuvant setting the patient was treated with doxorubicin/cyclophosphamide and taxol followed by tamoxifen. In March 2012, the patient received palliative radiation in the chest well. In Oct 2013, the patient's disease metastasized (T1/N1/M1) to the lung, liver, lymph nodes and bone. In the metastatic setting the patient received Fulvestrant/Lupron and Xgeva. NGS testing was done at MSKCC and the patient was found to have an *ERBB2* V777L mutation. In July 2014, the 37-year-old female Asian patient entered the SUMMIT study. On the SUMMIT study, the patient received and was maintained on 240 mg/day neratinib monotherapy and was on treatment for 166 days.

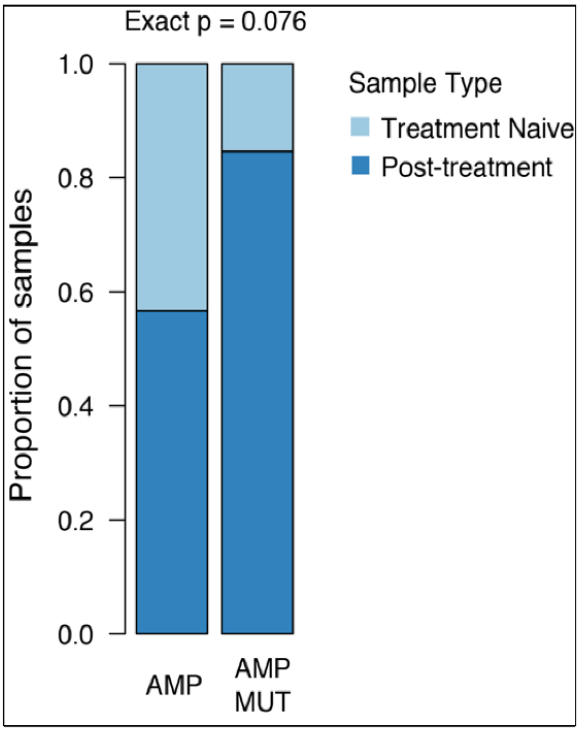

Fig. S1. Prevalence of coincident HER2 amplification and mutation in the MSKCC breast cancer cohort. The proportion of HER2-amplified only (AMP) and coincident HER2-amplified/HER2-mutated (AMP MUT) in the Memorial Sloan Kettering Cancer Center cohort of breast cancer patients that had not yet received therapy (light blue; AMP n=74 and AMP MUT n=2) or that had been treated (dark blue; AMP n=97 and AMP MUT n=11). Fisher exact p value.