



Evaluation of ANG/TIE/hypoxia pathway genes and signatures as predictors of response to trebananib (AMG 86) in the neoadjuvant I-SPY 2 TRIAL for Stage II-III high-risk breast cancer

Denise M Wolf^{1*}, Christina Yau^{1*}, Lamorna Brown-Swiger¹, Gillian Hirst¹, I-SPY 2 TRIAL Investigators, Smita Asare², Richard Schwab³, Don Berry⁴, Laura Esserman¹, Kathy Albain⁵, Brian Leyland-Jones⁶, Laura van 't Veer¹,
¹University of California San Francisco, ²QuantumLeap Healthcare Collaborative, ³The University of California San Diego, ⁴Berry Consultants, LLC, ⁵Loyola University, ⁶Avera Cancer Institute, *Equal contribution



I-SPY2 Trial

1. Hypothesis: We hypothesized that genes/signatures in the ANG/TIE signaling axis specifically predict response to angiogenesis (ANG1/2) inhibition, and that hypoxic tumors with a fragile blood supply are especially vulnerable to drugs in this class.

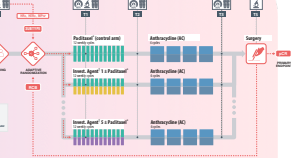
ANG1/2 inhibition
 Trebananib

- Biomarkers tested: 11 genes: TIE1/2, ANGPT1/2/4, AGNPTL1/3, VEGFA, ICAM1, PECAM1 and MMP2; and 2 signatures: hypoxia [PMC1334226] and angiogenesis (GO:001525).



2. THE PATIENTS: I-SPY 2 TRIAL Standing Platform

- Phase II, adaptively-randomized neoadjuvant trial
- Shared control arm Standard neoadjuvant chemotherapy
- Simultaneous experimental arms Up to four
- Primary endpoint: pathologic complete response (pCR) Defined as no residual invasive cancer in breast or lymph nodes
- Match therapies with most responsive breast cancer subtypes Defined by HR, HER2, and Mammprint High1(Ultra)High2 (MPT2) status
- Agents/combinations "graduate" for efficacy = reaching >95% predictive probability of success in a subsequent phase III trial in the most responsive patient subset
- Biomarker component:** evaluate biomarkers associated with mechanism of action of each agent, along with the pre-defined subsets



The ANG1/2 inhibitor AMG386 (trebananib, TR) was one of the experimental agents evaluated in I-SPY 2. It did not reach the graduation threshold, but within the TN subset appeared to perform better than control.

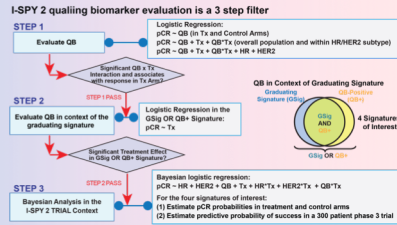
Subtype	Control arm (n=132)	AMG386 arm (n=134)
HR+HER2	15% (7/46)	16% (10/62)
HR+HER2 (TN)	20% (11/54)	43% (29/67)
HR+HER2+	42% (51/122)	50% (24)
HR+HER2+(TP)	16% (3/19)	27% (4/15)

3. DATA: Gene expression microarrays

Subtype	Control arm (n=132)	trebananib arm (n=134)	Total (n=266)
HR+HER2	47	52	100
HR+HER2 (TN)	54	53	107
HR+HER2+	12	4	16
HR+HER2+(TP)	19	15	34

Data from 266 patients (TR: 134 and concurrent controls: 132) were available. Pre-treatment biopsies were assayed using Agilent 44K (35227) or 32K (15746) expression arrays, and these data were combined using ComBat.

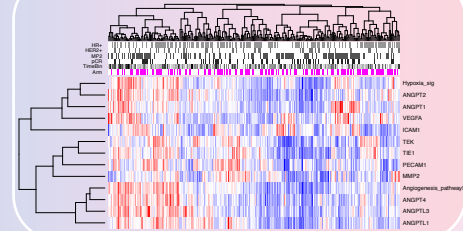
4. METHODS: Qualifying Biomarker Evaluation (QBE)



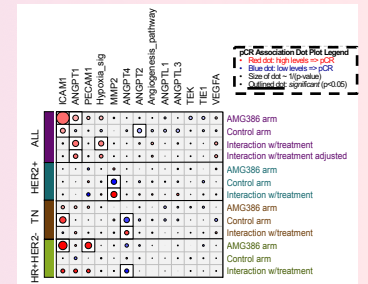
- All I-SPY 2 qualifying biomarker analyses follow a pre-specified analysis plan.
- We used logistic modeling to assess biomarker performance.
- A biomarker is considered a specific predictor of TR response if it associates with response in the TR arm but not the control arm, and if the biomarker x treatment interaction is significant (likelihood ratio test, p<0.05).
- This analysis is also performed adjusting for HR and HER2 status as covariates, and within receptor subtypes, sample size permitting.
- Our statistics are descriptive rather than inferential and do not adjust for multiplicities of other biomarkers outside this study.

5. RESULTS: Association between ANG/TIE pathway genes and hypoxia/angiogenesis signatures, and response to the ANG1/2 inhibitor trebananib (AMG386)

A. Unsupervised clustering heatmap



B. Association with response, by arm and receptor subset



- ANGPT1, a direct target of trebananib, associates with pCR in the TR arm but not the control arm, and shows a significant interaction with treatment that retains significance in a model adjusting for HR and HER2.
- ICAM1, expressed on endothelial and immune cells, associates with response in the TR arm, but also in the control arm in the population as a whole.
- In the HR+HER2+ subset, both ICAM1 and PECAM1 associate with pCR in the TR arm and not the control arm, with a trend toward interaction.

C. Exploratory analysis: Immune signaling, not ANG1/2 pathway or hypoxia, predicts response in the TN subset

- In the TN subset, where pCR rates were highest in the TR arm relative to control, these mechanism-of-action biomarkers fail to predict response.
- Rather, in exploratory whole genome analysis, response of TN's strongly associates with immune related genes and pathways (e.g. HLA's, IL21R, CCL13).

Category	Gene	Value	Gene	Value	Log Likelihood Ratio Test	Benjamini-Hochberg	QBE
ICAM1	ICAM1	0.00000000	ICAM1	0.00000000	0.0000	0.0000	0.0000
PECAM1	PECAM1	0.00000000	PECAM1	0.00000000	0.0000	0.0000	0.0000
ANGPT1	ANGPT1	0.00000000	ANGPT1	0.00000000	0.0000	0.0000	0.0000
ANGPT2	ANGPT2	0.00000000	ANGPT2	0.00000000	0.0000	0.0000	0.0000
ANGPT3	ANGPT3	0.00000000	ANGPT3	0.00000000	0.0000	0.0000	0.0000
ANGPT4	ANGPT4	0.00000000	ANGPT4	0.00000000	0.0000	0.0000	0.0000
ANGPT5	ANGPT5	0.00000000	ANGPT5	0.00000000	0.0000	0.0000	0.0000
ANGPT6	ANGPT6	0.00000000	ANGPT6	0.00000000	0.0000	0.0000	0.0000
ANGPT7	ANGPT7	0.00000000	ANGPT7	0.00000000	0.0000	0.0000	0.0000
ANGPT8	ANGPT8	0.00000000	ANGPT8	0.00000000	0.0000	0.0000	0.0000
ANGPT9	ANGPT9	0.00000000	ANGPT9	0.00000000	0.0000	0.0000	0.0000
ANGPT10	ANGPT10	0.00000000	ANGPT10	0.00000000	0.0000	0.0000	0.0000
ANGPT11	ANGPT11	0.00000000	ANGPT11	0.00000000	0.0000	0.0000	0.0000
ANGPT12	ANGPT12	0.00000000	ANGPT12	0.00000000	0.0000	0.0000	0.0000
ANGPT13	ANGPT13	0.00000000	ANGPT13	0.00000000	0.0000	0.0000	0.0000
ANGPT14	ANGPT14	0.00000000	ANGPT14	0.00000000	0.0000	0.0000	0.0000
ANGPT15	ANGPT15	0.00000000	ANGPT15	0.00000000	0.0000	0.0000	0.0000
ANGPT16	ANGPT16	0.00000000	ANGPT16	0.00000000	0.0000	0.0000	0.0000
ANGPT17	ANGPT17	0.00000000	ANGPT17	0.00000000	0.0000	0.0000	0.0000
ANGPT18	ANGPT18	0.00000000	ANGPT18	0.00000000	0.0000	0.0000	0.0000
ANGPT19	ANGPT19	0.00000000	ANGPT19	0.00000000	0.0000	0.0000	0.0000
ANGPT20	ANGPT20	0.00000000	ANGPT20	0.00000000	0.0000	0.0000	0.0000
ANGPT21	ANGPT21	0.00000000	ANGPT21	0.00000000	0.0000	0.0000	0.0000
ANGPT22	ANGPT22	0.00000000	ANGPT22	0.00000000	0.0000	0.0000	0.0000
ANGPT23	ANGPT23	0.00000000	ANGPT23	0.00000000	0.0000	0.0000	0.0000
ANGPT24	ANGPT24	0.00000000	ANGPT24	0.00000000	0.0000	0.0000	0.0000
ANGPT25	ANGPT25	0.00000000	ANGPT25	0.00000000	0.0000	0.0000	0.0000
ANGPT26	ANGPT26	0.00000000	ANGPT26	0.00000000	0.0000	0.0000	0.0000
ANGPT27	ANGPT27	0.00000000	ANGPT27	0.00000000	0.0000	0.0000	0.0000
ANGPT28	ANGPT28	0.00000000	ANGPT28	0.00000000	0.0000	0.0000	0.0000
ANGPT29	ANGPT29	0.00000000	ANGPT29	0.00000000	0.0000	0.0000	0.0000
ANGPT30	ANGPT30	0.00000000	ANGPT30	0.00000000	0.0000	0.0000	0.0000
ANGPT31	ANGPT31	0.00000000	ANGPT31	0.00000000	0.0000	0.0000	0.0000
ANGPT32	ANGPT32	0.00000000	ANGPT32	0.00000000	0.0000	0.0000	0.0000
ANGPT33	ANGPT33	0.00000000	ANGPT33	0.00000000	0.0000	0.0000	0.0000
ANGPT34	ANGPT34	0.00000000	ANGPT34	0.00000000	0.0000	0.0000	0.0000
ANGPT35	ANGPT35	0.00000000	ANGPT35	0.00000000	0.0000	0.0000	0.0000
ANGPT36	ANGPT36	0.00000000	ANGPT36	0.00000000	0.0000	0.0000	0.0000
ANGPT37	ANGPT37	0.00000000	ANGPT37	0.00000000	0.0000	0.0000	0.0000
ANGPT38	ANGPT38	0.00000000	ANGPT38	0.00000000	0.0000	0.0000	0.0000
ANGPT39	ANGPT39	0.00000000	ANGPT39	0.00000000	0.0000	0.0000	0.0000
ANGPT40	ANGPT40	0.00000000	ANGPT40	0.00000000	0.0000	0.0000	0.0000
ANGPT41	ANGPT41	0.00000000	ANGPT41	0.00000000	0.0000	0.0000	0.0000
ANGPT42	ANGPT42	0.00000000	ANGPT42	0.00000000	0.0000	0.0000	0.0000
ANGPT43	ANGPT43	0.00000000	ANGPT43	0.00000000	0.0000	0.0000	0.0000
ANGPT44	ANGPT44	0.00000000	ANGPT44	0.00000000	0.0000	0.0000	0.0000
ANGPT45	ANGPT45	0.00000000	ANGPT45	0.00000000	0.0000	0.0000	0.0000
ANGPT46	ANGPT46	0.00000000	ANGPT46	0.00000000	0.0000	0.0000	0.0000
ANGPT47	ANGPT47	0.00000000	ANGPT47	0.00000000	0.0000	0.0000	0.0000
ANGPT48	ANGPT48	0.00000000	ANGPT48	0.00000000	0.0000	0.0000	0.0000
ANGPT49	ANGPT49	0.00000000	ANGPT49	0.00000000	0.0000	0.0000	0.0000
ANGPT50	ANGPT50	0.00000000	ANGPT50	0.00000000	0.0000	0.0000	0.0000
ANGPT51	ANGPT51	0.00000000	ANGPT51	0.00000000	0.0000	0.0000	0.0000
ANGPT52	ANGPT52	0.00000000	ANGPT52	0.00000000	0.0000	0.0000	0.0000
ANGPT53	ANGPT53	0.00000000	ANGPT53	0.00000000	0.0000	0.0000	0.0000
ANGPT54	ANGPT54	0.00000000	ANGPT54	0.00000000	0.0000	0.0000	0.0000
ANGPT55	ANGPT55	0.00000000	ANGPT55	0.00000000	0.0000	0.0000	0.0000
ANGPT56	ANGPT56	0.00000000	ANGPT56	0.00000000	0.0000	0.0000	0.0000
ANGPT57	ANGPT57	0.00000000	ANGPT57	0.00000000	0.0000	0.0000	0.0000
ANGPT58	ANGPT58	0.00000000	ANGPT58	0.00000000	0.0000	0.0000	0.0000
ANGPT59	ANGPT59	0.00000000	ANGPT59	0.00000000	0.0000	0.0000	0.0000
ANGPT60	ANGPT60	0.00000000	ANGPT60	0.00000000	0.0000	0.0000	0.0000
ANGPT61	ANGPT61	0.00000000	ANGPT61	0.00000000	0.0000	0.0000	0.0000
ANGPT62	ANGPT62	0.00000000	ANGPT62	0.00000000	0.0000	0.0000	0.0000
ANGPT63	ANGPT63	0.00000000	ANGPT63	0.00000000	0.0000	0.0000	0.0000
ANGPT64	ANGPT64	0.00000000	ANGPT64	0.00000000	0.0000	0.0000	0.0000
ANGPT65	ANGPT65	0.00000000	ANGPT65	0.00000000	0.0000	0.0000	0.0000
ANGPT66	ANGPT66	0.00000000	ANGPT66	0.00000000	0.0000	0.0000	0.0000
ANGPT67	ANGPT67	0.00000000	ANGPT67	0.00000000	0.0000	0.0000	0.0000
ANGPT68	ANGPT68	0.00000000	ANGPT68	0.00000000	0.0000	0.0000	0.0000
ANGPT69	ANGPT69	0.00000000	ANGPT69	0.00000000	0.0000	0.0000	0.0000
ANGPT70	ANGPT70	0.00000000	ANGPT70	0.00000000	0.0000	0.0000	0.0000
ANGPT71	ANGPT71	0.00000000	ANGPT71	0.00000000	0.0000	0.0000	0.0000
ANGPT72	ANGPT72	0.00000000	ANGPT72	0.00000000	0.0000	0.0000	0.0000
ANGPT73	ANGPT73	0.00000000	ANGPT73	0.00000000	0.0000	0.0000	0.0000
ANGPT74	ANGPT74	0.00000000	ANGPT74	0.00000000	0.0000	0.0000	0.0000
ANGPT75	ANGPT75	0.00000000	ANGPT75	0.00000000	0.0000	0.0000	0.0000
ANGPT76	ANGPT76	0.00000000	ANGPT76	0.00000000	0.0000	0.0000	0.0000
ANGPT77	ANGPT77	0.00000000	ANGPT77	0.00000000	0.0000	0.0000	0.0000
ANGPT78	ANGPT78	0.00000000	ANGPT78	0.00000000	0.0000	0.0000	0.0000
ANGPT79	ANGPT79	0.00000000	ANGPT79	0.00000000	0.0000	0.0000	0.0000
ANGPT80	ANGPT80	0.00000000	ANGPT80	0.00000000	0.0000	0.0000	0.0000
ANGPT81	ANGPT81	0.00000000	ANGPT81	0.00000000	0.0000	0.0000	0.0000
ANGPT82	ANGPT82	0.00000000	ANGPT82	0.00000000	0.0000	0.0000	0.0000
ANGPT83	ANGPT83	0.00000000	ANGPT83	0.00000000	0.0000	0.0000	0.0000
ANGPT84	ANGPT84	0.00000000	ANGPT84	0.00000000	0.0000	0.0000	0.0000
ANGPT85	ANGPT85	0.00000000	ANGPT85	0.00000000	0.0000	0.0000	0.0000
ANGPT86	ANGPT86	0.00000000	ANGPT86	0.00000000	0.0000	0.0000	0.0000
ANGPT87	ANGPT87	0.00000000	ANGPT87	0.00000000	0.0		