

Heterogeneity of Mutated Tumor Antigens In A Single High Grade Ovarian Serous Carcinoma



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Overview

Patient PT189 presented with stage IIIC high grade papillary serous ovarian cancer in 2012. She was originally treated with carboplatin/paclitaxel. Having failed this original doublet chemotherapy, she has received a total of five additional chemotherapy regimens, all with recurrence of her disease.

We performed exome and RNA sequencing on normal PBMC as well as nine tumor samples collected over a two year period during the course of her treatment.

Predicting neoepitopes from PT189's sequence data revealed a diversity of results across samples and choice of variant calling algorithm.

Bioinformatics

DNA

- Alignment to hg19 with BWA-mem
- Post-processing: BQSR and IndelRealigner
- Variant calling with Mutect and Strelka

RNA

- Alignment to hg19 with STAR
- Transcript abundance estimation: Cufflinks

Mutant Epitope Prediction

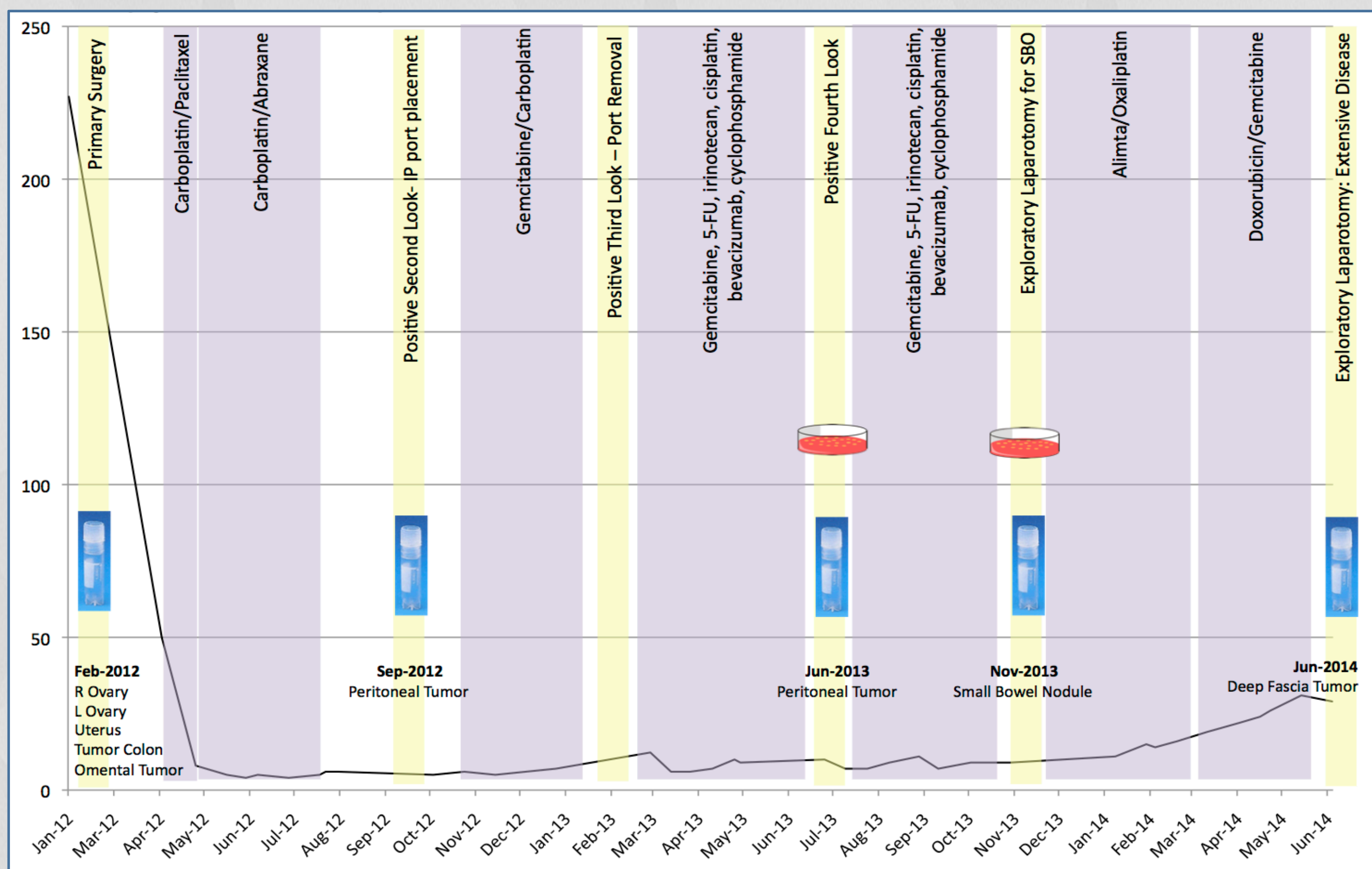
- Protein sequence prediction: Vicode
- MHC binding prediction: NetMHCpan
- Epitope lengths: 8-11
- Affinity filter: $\leq 500\text{nM}$, $\leq 5^{\text{th}}$ percentile
- Transcript abundance filter: 0.1 FPKM

HLA Typing

- RNA: seq2HLA
- DNA: OptiType

Summary

- Choice of variant calling algorithm yields significantly different results in each sample
- Low overlap between epitope predictions for primary vs. recurrent sample sets using "confident" variants
- Greater concordance between samples from the primary time point compared with later recurrences
- seq2hla and OptiType agree on patient HLA type on all tumor samples



Predicted MHC Class I Epitopes

Variant Caller \ # Epitopes	Primary Right Ovary	Primary Omentum	Primary Colon	Primary Uterus	Recurrence Peritoneal (+7 months)	Recurrence Peritoneal (+16 months)	Recurrence Small Bowel (+21 months)	Recurrence Deep Fascia (+28 months)
Mutect, NetMHCpan	78	34	53	44	8	43	42	45
Strelka, NetMHCpan	28	30	17	20	4	9	17	13
Mutect & Strelka, NetMHCpan	16	28	13	18	0	4	11	13

PT189 Alleles: A*02:01, A*24:02, B*27:05, B*35:01, C*02:02, C*04:01

Variant Calls

Variant Caller \ # SNVs	Primary Right Ovary	Primary Omentum	Primary Colon	Primary Uterus	Recurrence Peritoneal (+7 months)	Recurrence Peritoneal (+16 months)	Recurrence Small Bowel (+21 months)	Recurrence Deep Fascia (+28 months)
Mutect	88	57	286	46	33	35	38	73
Strelka	27	23	20	19	6	12	17	17
Mutect & Strelka	19	16	13	13	2	5	9	10



Overlap across samples of mutations detected by both Mutect and Strelka



Overlap across samples of predicted epitopes derived from variants detected by both Mutect and Strelka