Causal Modeling of CALGB/SWOG 80405 (Alliance) Identifies Primary (1°) Side-related Angiogenic Drivers of Metastatic Colorectal Cancer (mCRC)

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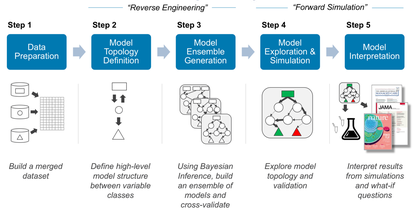
ABSTRACT

- CALGB/SWOG 80405 was a phase III clinical trial of FOLFOX or FOLFIRI with randomly assigned cetuximab (Cet) or bevacizumab (Bev) in metastatic CRC (mCRC) patients.
- Retrospective analysis of CALGB 80405 data revealed that side of the primary tumor is both a prognostic factor of OS and PFS and a predictive factor of response to Bev and Cet.
- None of the existing sub-classifications of CRC, which are based on genetic mutations or gene-expression data, have defined a right- or left-sided tumor.
- We leveraged hypothesis-free Bayesian machine learning approach to build a multivariate causal models of mCRC survival (OS) and examined the network drivers of OS.
- The molecular underpinnings of sidedness in the mCRC patients were uncovered. An angiogenesis/extracellular matrix (ECM) remodeling gene signature was found to be a negative prognostic factor for OS, more prevalent in right-tumors and a potential predictive biomarker of response to Bev.

METHODS

Fig. 1: Schematic of REFS™ Reverse Engineering & Forward Simulation Workflow



- Using our Bayesian causal machine learning platform REFS, an ensemble of 128 network models were built for overall survival (OS) of mCRC.
- The ensemble enables estimation of model uncertainty and identification of key drivers by model consensus.
- Simulations were performed on the ensemble to identify causal drivers of OS after accounting for confounders. Causal effect was quantified by median hazard ratio (HR). For continuous variables, 3rd & 1st quartile values were used to compute HR.
- Analysis of NanoString data:
  - Molecular/gene clusters were computed using consensus clustering.
  - Consensus molecular subtypes (CMS) were computed using published code (Guinney et al., Nat. Med. 2015) on GitHub.
  - Molecular clusters and CMSs were used in modeling as proxies of gene expression data
- Patients with both KRAS wild-type and mutant tumors were included and those who received both Cet and Bev treatments were excluded.
- Independent cohorts were withheld and used for causal drivers validation.

CAUSAL MODELS

- Clinical variables only (N=1463, 68 variables)
- Clinical and molecular variables (N=461, 84 vars)

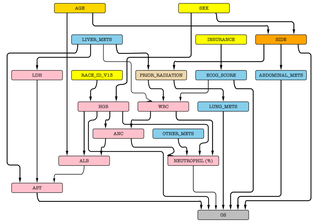
Clinical Data
Mutation
Microsatellite Instability
Gene Expression (Gene Clusters, CMS)

RESULTS

Clinical Causal Drivers of OS

- Side of the primary tumor was a direct and independent causal driver of OS when molecular data was not considered
  - consistent with the retrospective analysis of CALGB 80405 data.
- ECOG performance score, concentrations of aspartate aminotransferase (AST), hemoglobin (HGB), absolute neutrophil counts (ANC), lactate dehydrogenase (LDH) and metastases at intra-abdominal, lung, and liver were the other causal drivers of OS.

Fig. 2: Reverse Engineering: Consensus Subnetwork to OS



Three Molecular Clusters

Fig.3: Gene Expression Profiles of Molecular Clusters

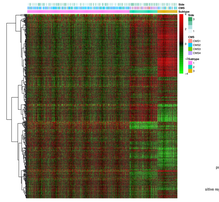
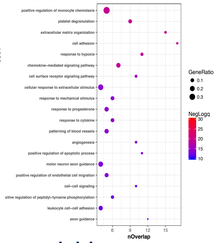


Fig. 4: Over-represented GO Biological Processes in Angiogenesis Gene Cluster



Clustering of NanoString data revealed three gene signatures with upregulation of different pathways:

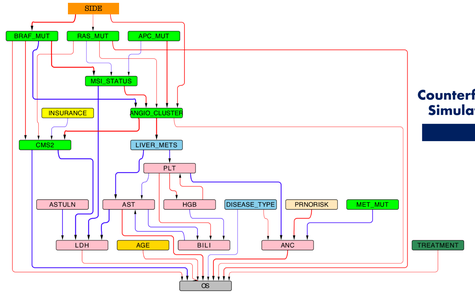
- Angiogenesis & extracellular matrix remodeling
- WNT-signaling
- Immune infiltration

- For model building, these molecular clusters were used as proxies of gene expression data

Molecular Causal Drivers of OS

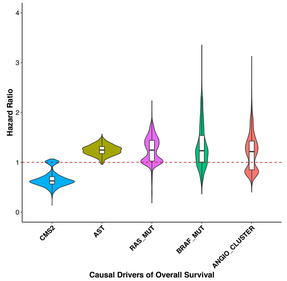
- Side of the primary tumor was not a direct driver of OS
  - Causal effects of 1° side on OS was found to be driven by molecular pathways
  - Key components of pathways: angiogenesis/ECM remodeling gene signature and BRAF mutation (V600E)
  - Angiogenesis/ECM remodeling signature, BRAF mutation, and RAS mutation were the top molecular causal drivers of OS.

Fig. 5: Reverse Engineering: Consensus Subnetwork to OS



Counterfactual Simulations

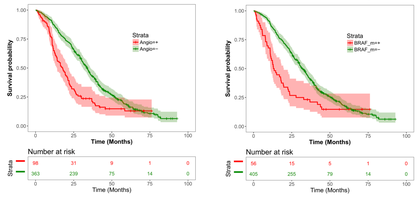
Fig. 6: Top Causal Drivers of OS



Side is a Surrogate of Non-random Distributions of Molecular Features

- Angiogenesis/ECM remodeling gene signature [HR = 2.1 (95% CI = 1.38-3.20), log-rank p = 4.0e-03] and BRAF mutation [HR = 2.99 (95% CI = 1.39-6.43), log-rank p = 0.003] were validated (N=168 and 79, respectively) as **negative** prognostic factors of OS.
- Prognostic effects were independent of the sidedness of the primary tumor.
- Angiogenesis gene signature (odds ratio = 3.5, p = 1.3e-07) and BRAF mutation (odds ratio = 7.2, p = 4.1e-10) were significantly more prevalent in the right sided tumors.
- These factors explain the poorer survival of right vs. left-sided tumors.

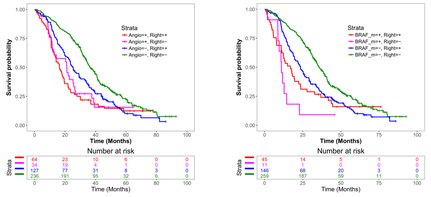
Fig. 7: Causal Drivers as Prognostic Factors of OS
univariate Kaplan Meier survival curves and risk tables (Training Cohort)



HR =1.78 (95% CI = 1.39-2.28)
Log-rank p = 3.2e-06

HR =1.79 (95% CI = 1.32-3.43)
Log-rank p = 1.6e-04

Fig. 8: Prognostic Effect is Independent of Side
univariate Kaplan Meier survival curves and risk tables (Training Cohort)



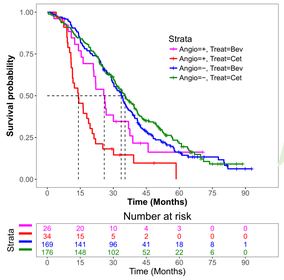
Angio +ve, Right vs. Angio +ve, Left
Log-rank p = 0.52 (Training), 0.98 (Validation)

BRAF mutant, Right vs. BRAF mutant, Left
Log-rank p = 0.21

Angiogenesis Gene Signature: Predictive Biomarker

- Patients lacking angiogenesis signature didn't show any difference in response to treatment with Cet vs. Bev (log-rank p = 0.3).
- Patients with upregulation of angiogenesis/ECM remodeling gene signature were benefitted from Bevacizumab (log-rank p = 0.02).

Fig. 9: Predictive effect of Angiogenesis Signature
(Training Cohort: BRAF WT)



- The median OS was 11.7 months longer[+] for angiogenesis signature rich patients when treated with Bev vs. Cet[+]
- As patients with right-sided tumors are more likely to be angiogenesis signature-rich, this explains why they responded to Bev but not Cet

[+]The median OS was 8.4 months longer in the validation cohort (N=64) but the KM curves for angiogenesis rich patients were not statistically different between Cet and Bev arms due to small sample size of these strata

CONCLUSIONS

- Hypothesis-free Bayesian causal modeling identified an angiogenesis/extracellular matrix remodeling gene signature and BRAF mutation (V600E) as causal drivers of OS in mCRC. Both accounted for poor prognosis.
- Molecular pathways comprising angiogenesis signature and BRAF mutation were identified between side of primary tumor and OS.
- Angiogenesis gene signature and BRAF mutation were more prevalent in right vs. left-sided tumors, thus explaining why right tumors have poorer survival.
- Angiogenesis signature was found to be a potential predictive factor of response to Bevacizumab. As patients with right-sided tumors are more likely to be angiogenesis signature rich, this explains why they responded to Bev but not Cetuximab.
- Measurements of these prognostic and predictive factors at baseline will enable making better personalized decisions for treating right- or left-sided tumors.
- Additional research is necessary to confirm these findings in a larger and independent cohort.

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