Biomarkers to Inform Clinical Medicine – A Case Study

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Target Disease for the biomarker

- Newly diagnosed glioblastoma
- Mean survival 12-14 months from diagnosis
- Mean survival 4-5 months from recurrence
- 2 year survival 10%
- Recurrence occurs within 2-3 cm of the margins of the original tumor in 90% of patients

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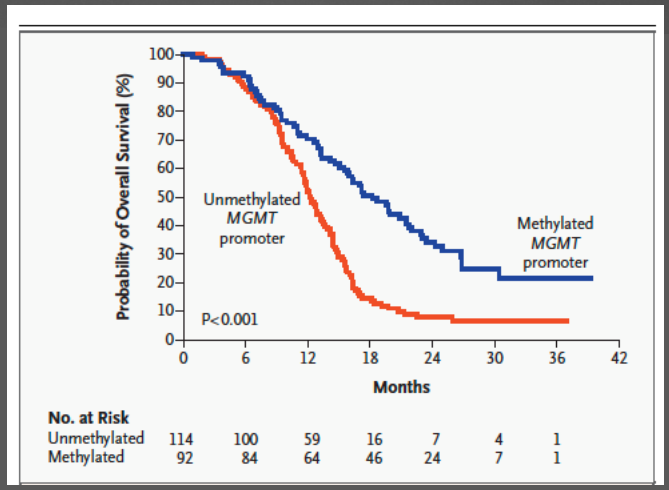
Current Biomarker for GBM: MGMT



Methyl-MGMT (good prognosis)	Un-Methyl-MGMT (poor prognosis)
OS= 22 Months	OS= 12 Months
70% (1 year survival)	52% (1 year survival)

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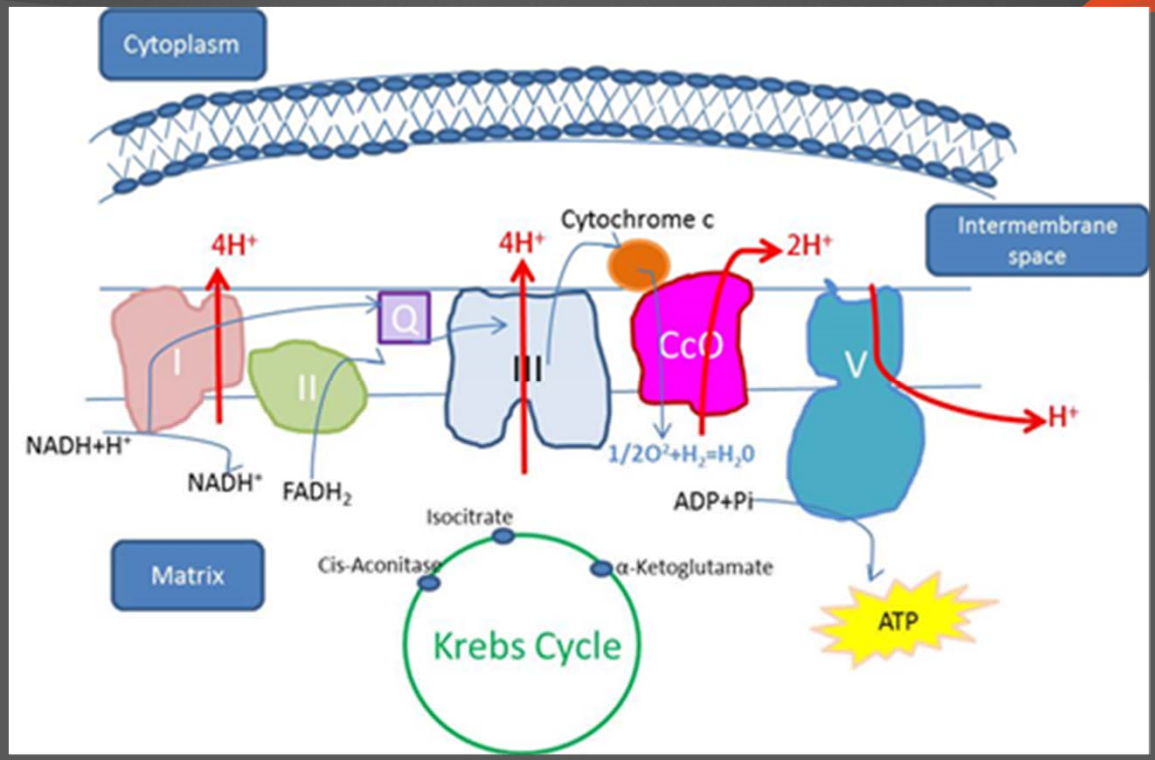
Cytochrome c Oxidase: Inside the mitochondria



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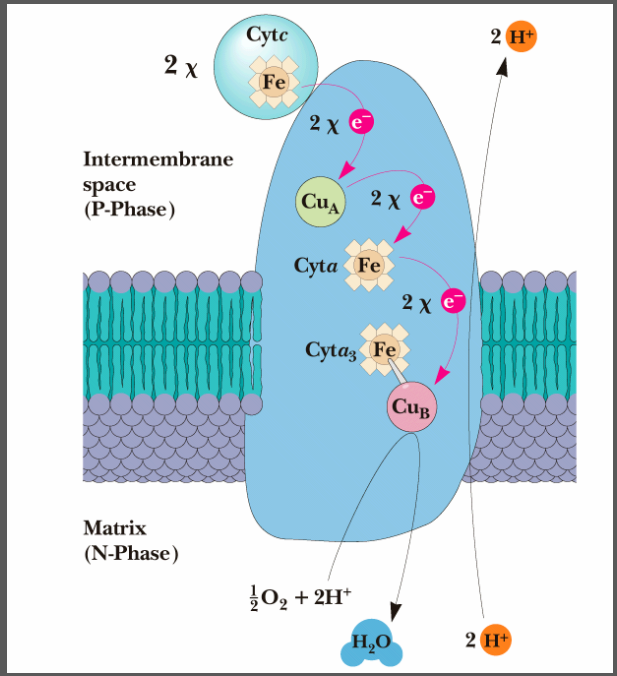
Cytochrome C Oxidase (CcO)

- Catalyzes the reduction of oxygen by reduced cytochrome c
- 13 subunits, 10 encoded by nDNA and 3 by mtDNA
- All catalytic subunits are coded by Mt DNA
- Dysfunction of CcO structure/function are the most severe mt related diseases
- Metabolic disorders in early childhood (Brain, Heart and Muscle)
  - Leigh Syndrome
  - Hypertrophic Cardiomyopathy
  - Myopathy



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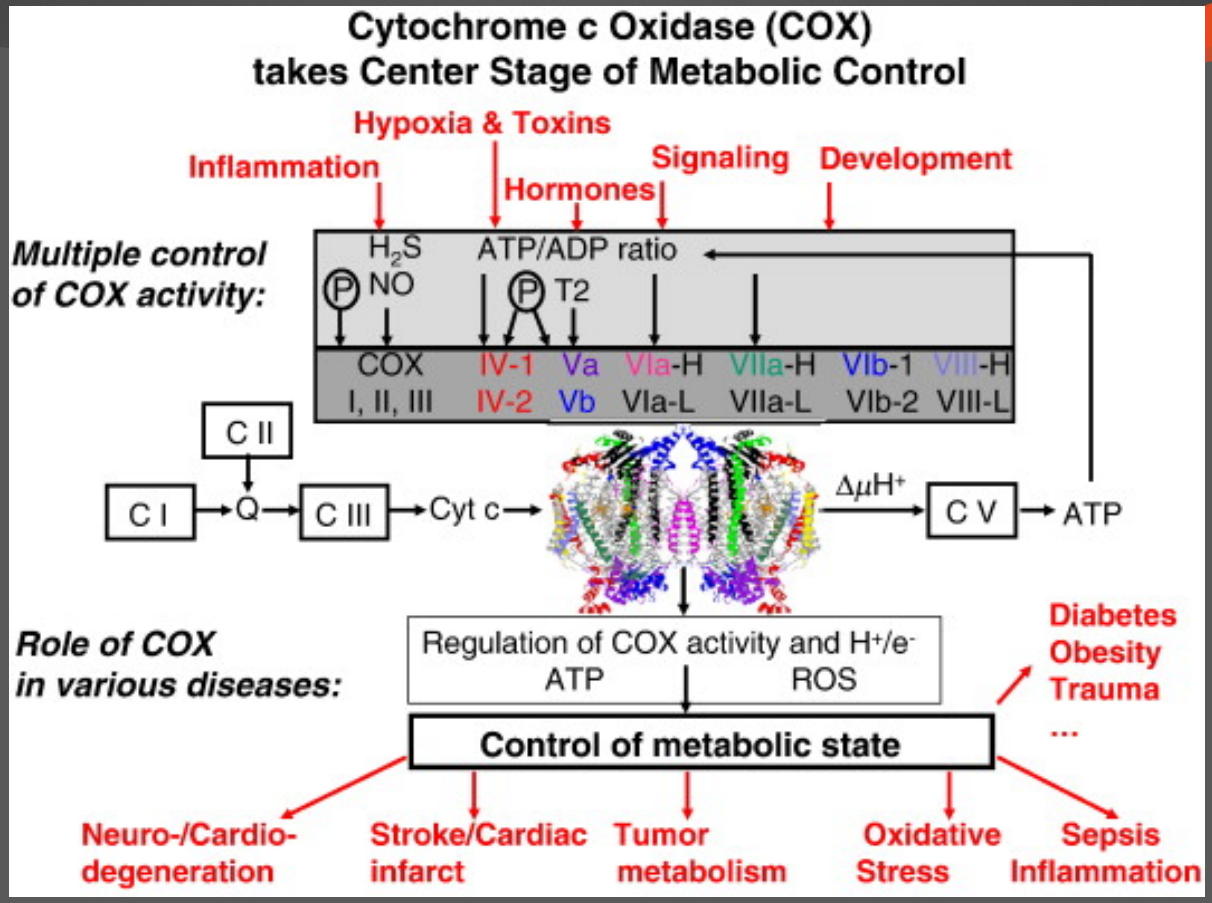

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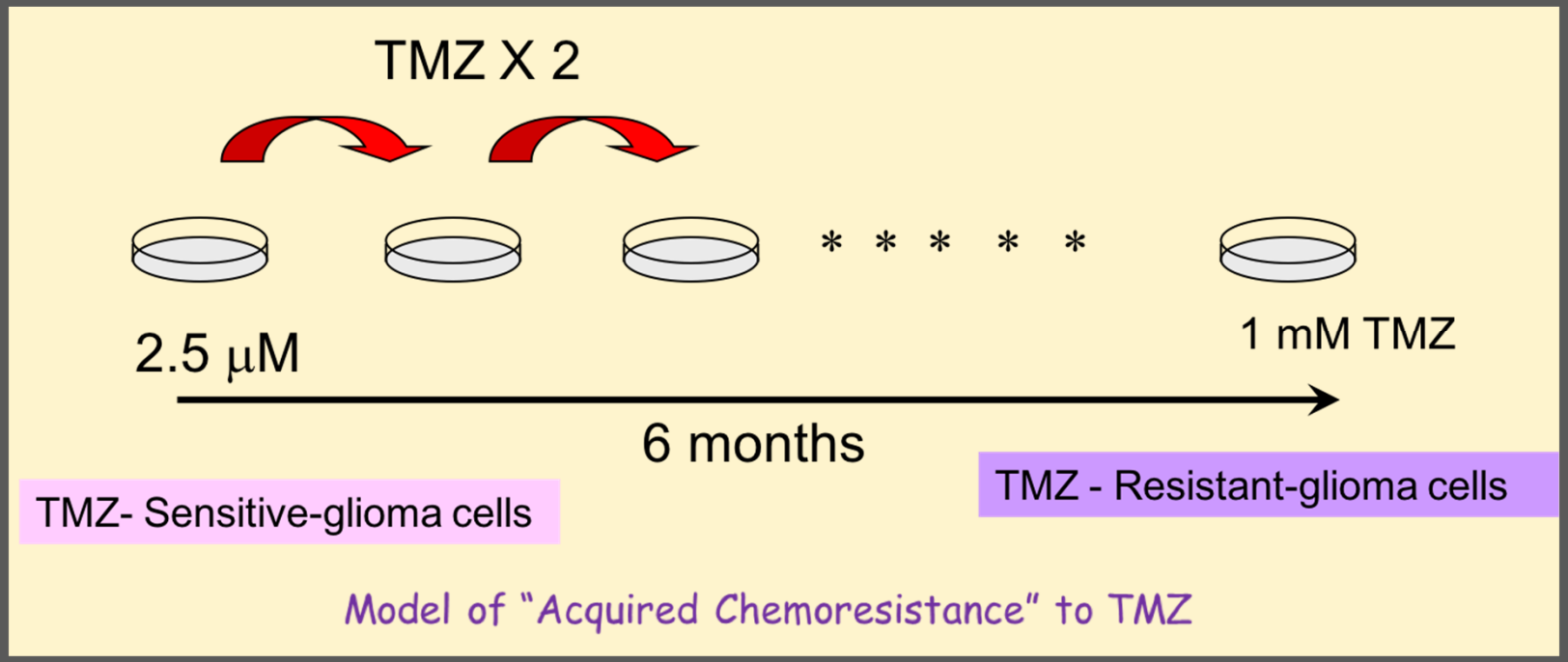
Generation of TMZ Resistant Human Glioma Cells



Model of "Acquired Chemoresistance" to TMZ

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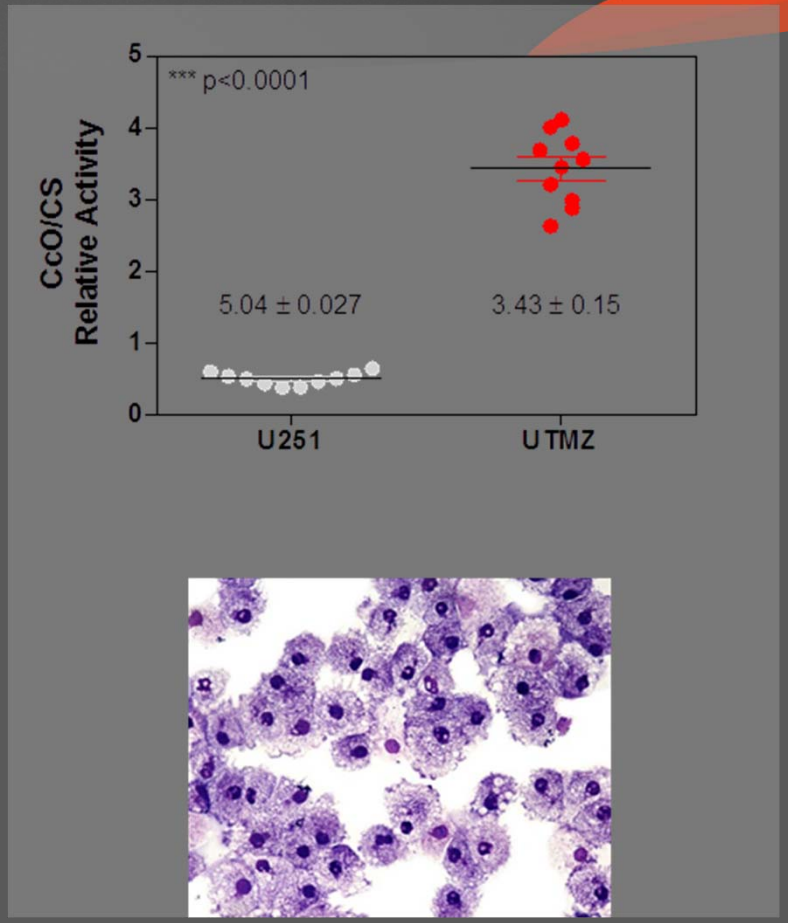
Evidences that Cytochrome C Oxidase activity is associated with poor outcome in glioblastoma patients



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Generation of TMZ Resistant Human Glioma Cells

TMZ sensitive tumor lines

GBM12 (Classical)
GBM14 (Classical)
GBM22 (Mesenchymal)
GBM39 (Classical)

TMZ-resistant tumor lines

GBM12TMZ (Classical)
GBM14TMZ (Classical)
GBM22TMZ (Mesenchymal)
GBM39TMZ (Neuronal)

flank tumors

TMZ x 2

Tumor growth unaffected by dosing with TMZ at 120 mg/kg/day for 5 days.

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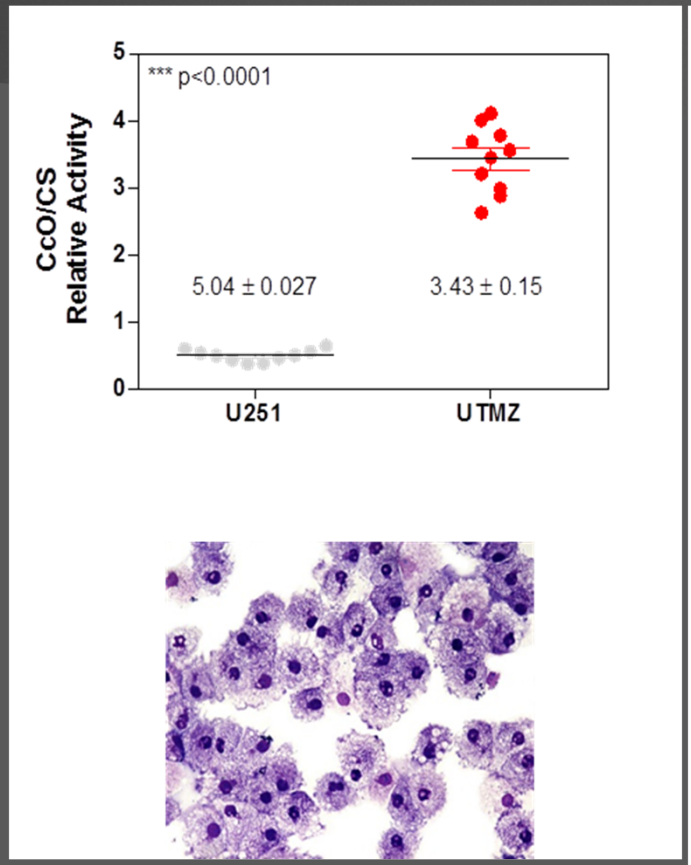

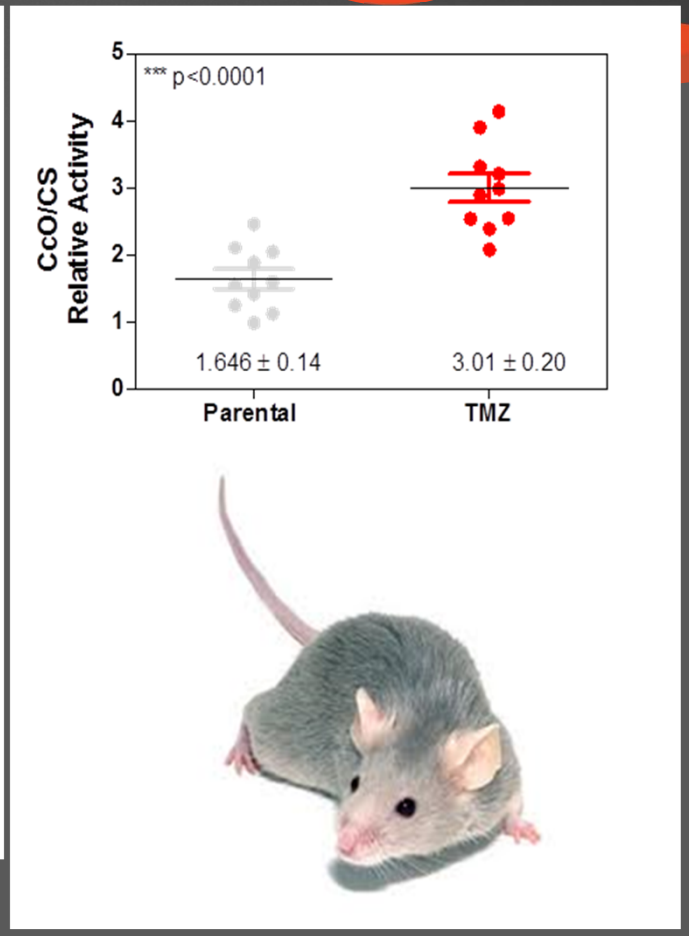

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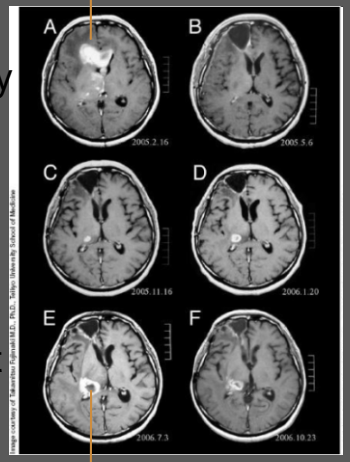
Tissue from GBM Patients

Primary

Recurrent



Tissues from First Diagnosis
NO Treatment

Tissues from Recurrent GBM
Radiotherapy and TMZ

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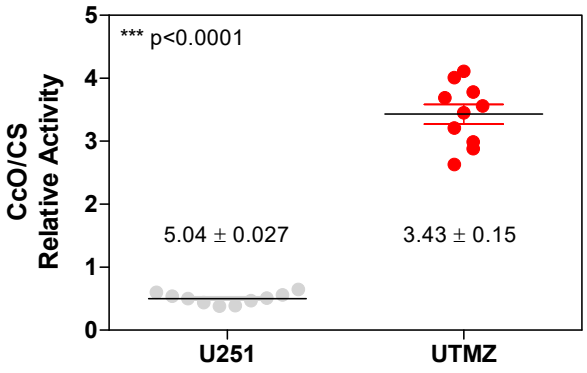
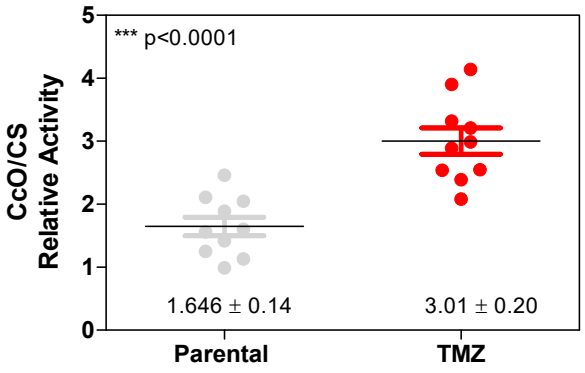
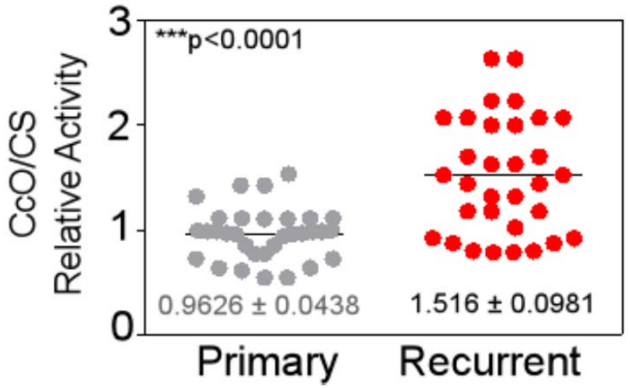
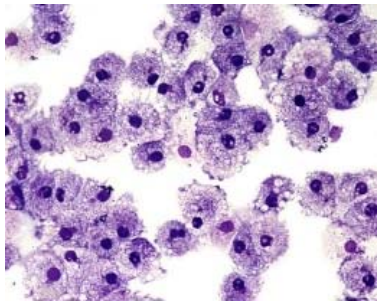
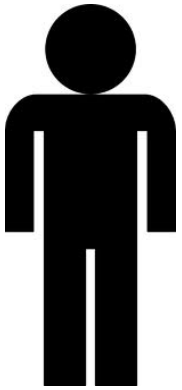

*** p<0.0001
CcO/CS
Relative Activity
5
4
3
2
1
0
5.04 ± 0.027
3.43 ± 0.15
U251
UTMZ


*** p<0.0001
CcO/CS
Relative Activity
5
4
3
2
1
0
1.646 ± 0.14
3.01 ± 0.20
Parental
TMZ

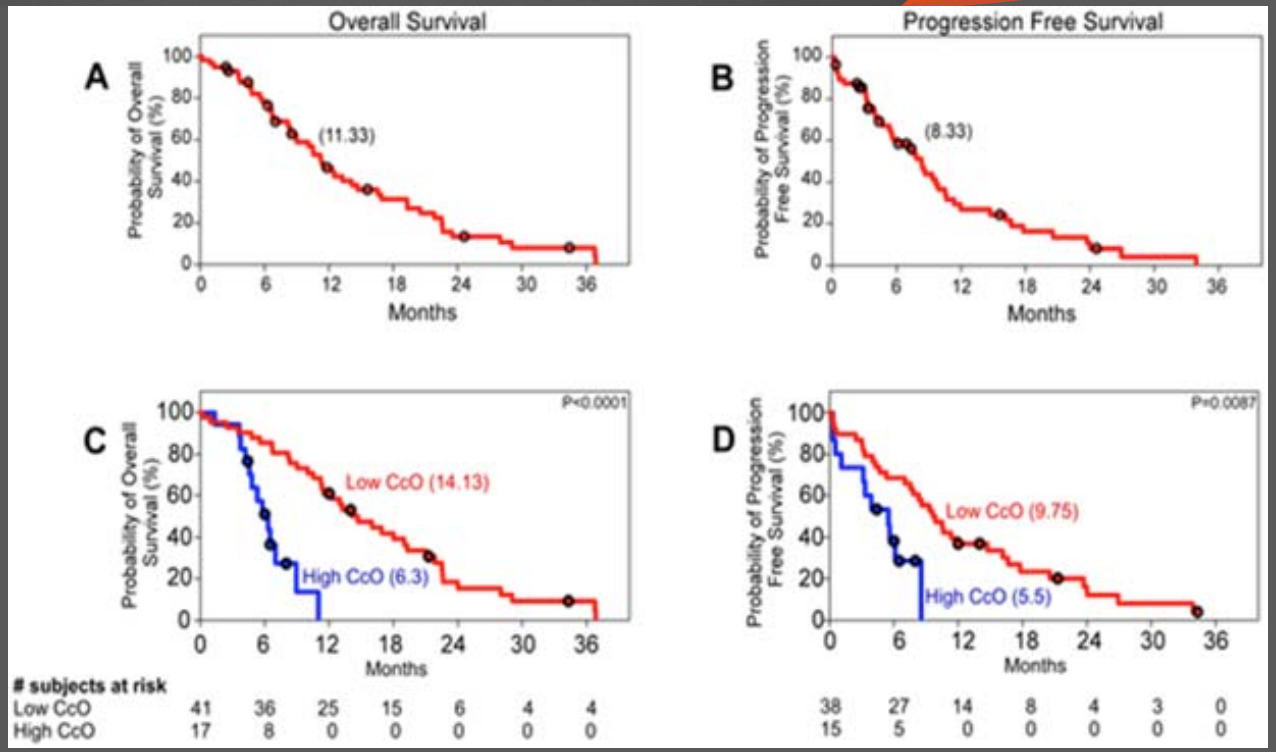

***p<0.0001
CcO/CS
Relative Activity
3
2
1
0
0.9626 ± 0.0438
1.516 ± 0.0981
Primary
Recurrent





Retrospective study

Training Set Using GBM Tumor Tissues from UAB Hospital



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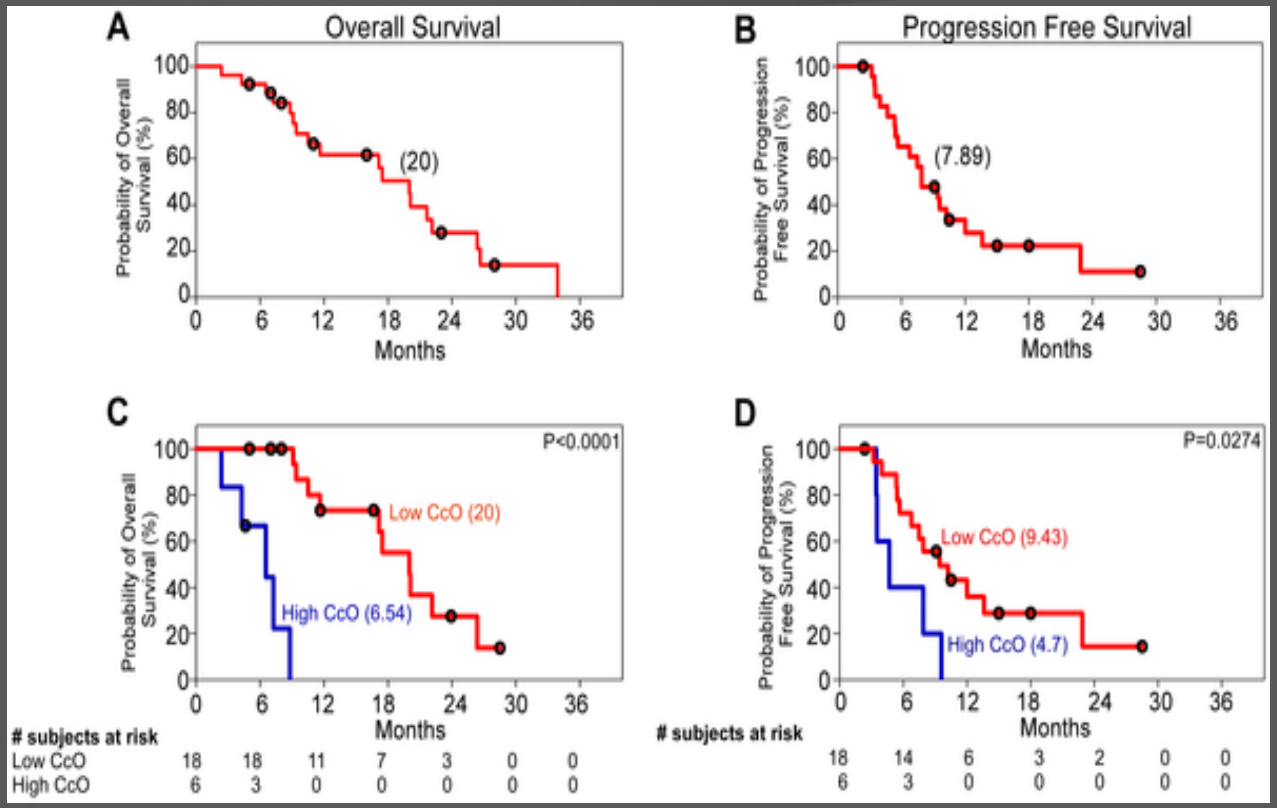
Retrospective Study

Validation Set from Geneva Hospital



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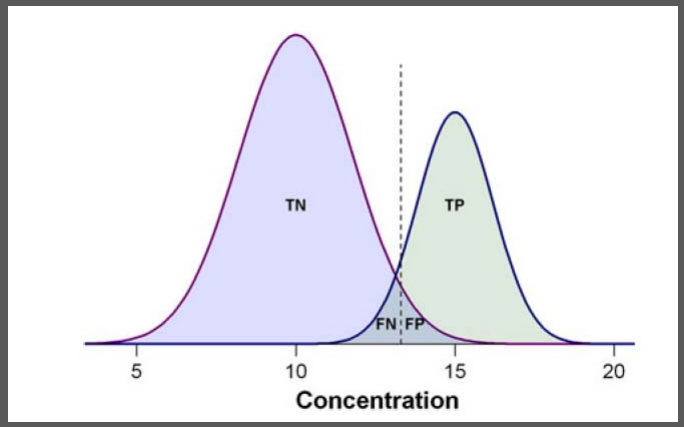
Main Criteria to Assess a Biomarker (1)



- TP the number of diseased subjects that are correctly identified as disease (outcome positive&test positive)
- TN the number of healthy subjects that are correctly identified as healthy (outcome negative & test negative)
- FP the number of healthy subjects that are incorrectly identified as diseased (outcome negative&test positive)
- FN the number of diseased subjects that are incorrectly identified as healthy (outcome positive&test negative)

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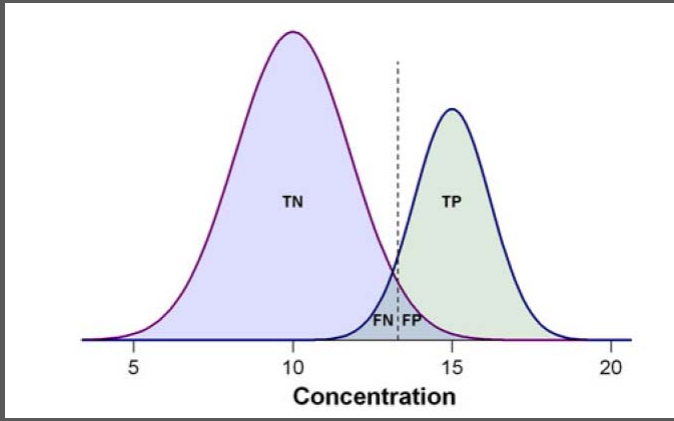
Main Criteria to Assess a Biomarker (2)



- Sensitivity = TP/(TP / FN)
- Specificity = TN/(TN / FP)
- Sensitivity can be considered as the probability of a positive test result given that a subject has an actual positive outcome
- Specificity can be considered as the probability of a negative test result given that a subject has an actual negative outcome

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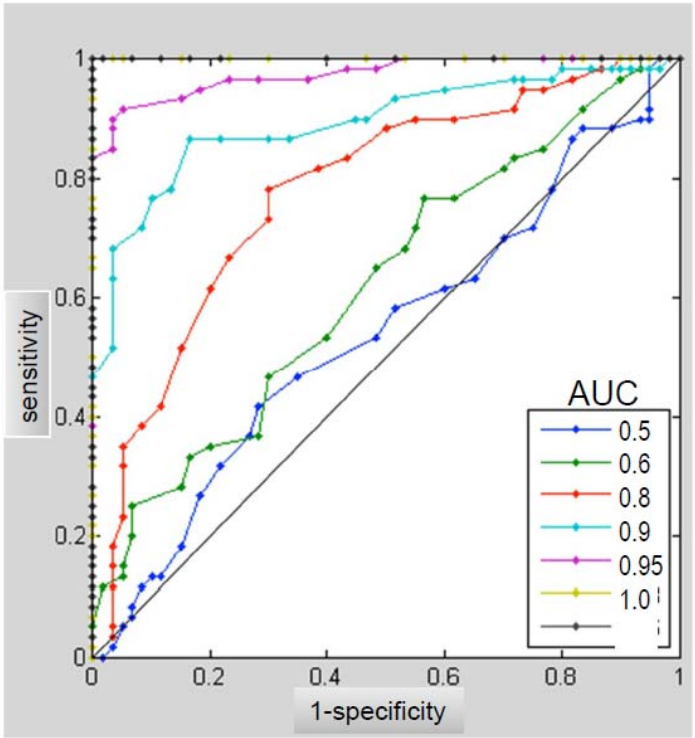
Area Under the Curve: Good or Bad



- Plots sensitivity (%TP) vs. specificity (%TN)
- A poor ROC curve would be a straight line with a slope of 1
- The area under an ROC (AUROC) curve is a good measure of the quality of the biomarker
- AUCs of >0.75 are good, AUCs of 0.5 are terrible, AUCs of 1.00 are perfect

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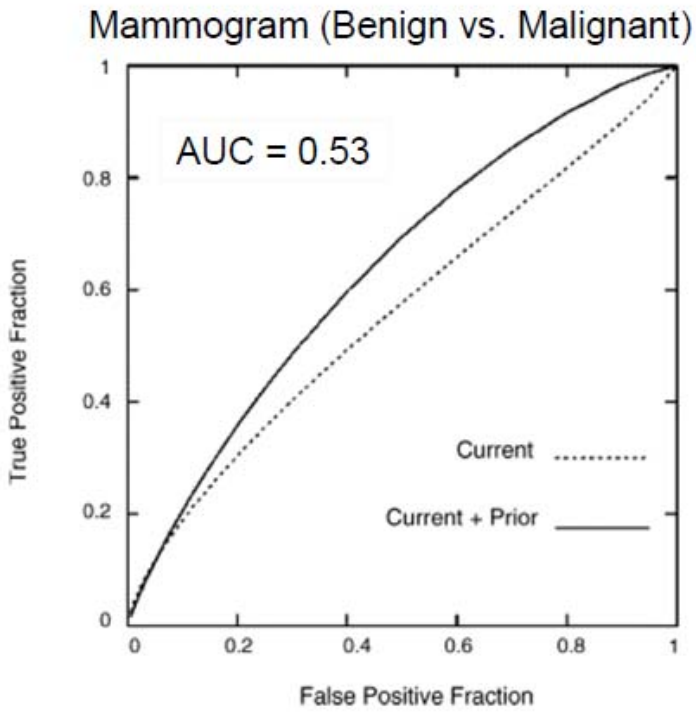
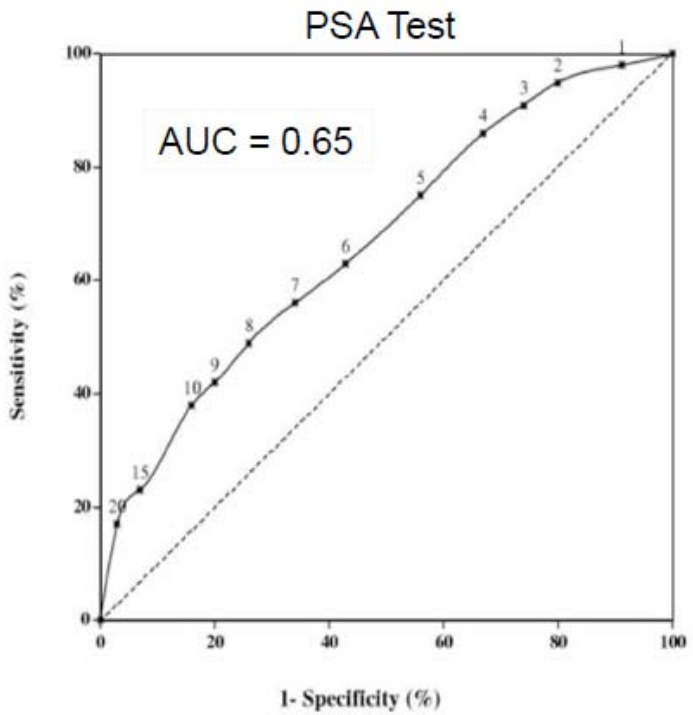
AUCs of Common Tests





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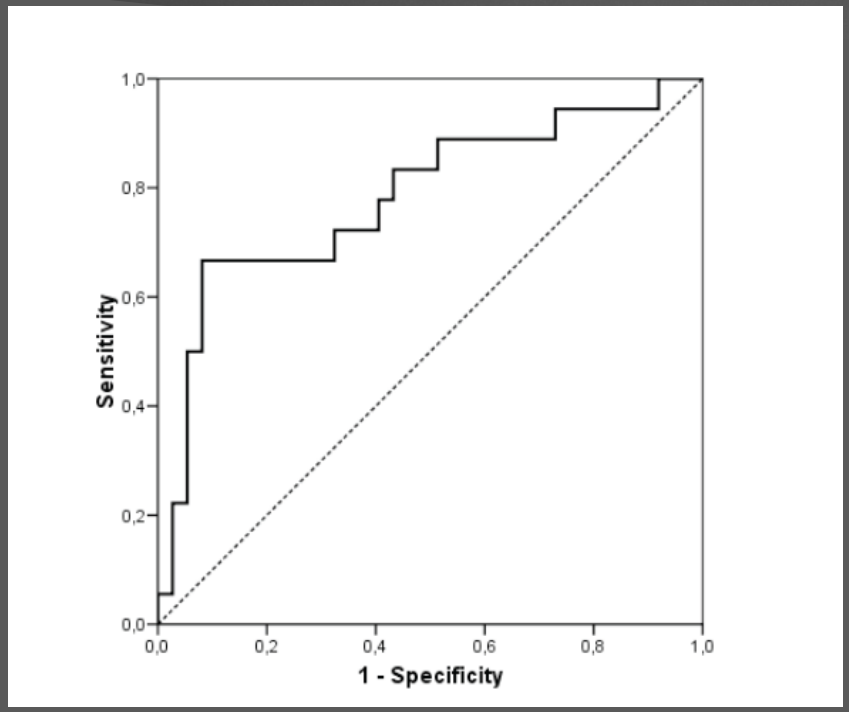
IDH Mutation in Glioma



ROC Curve:

- Sensitivity 0.76
- Specificity 0.8
- AUC 0.8

50 patients

Lombardi G, 2015

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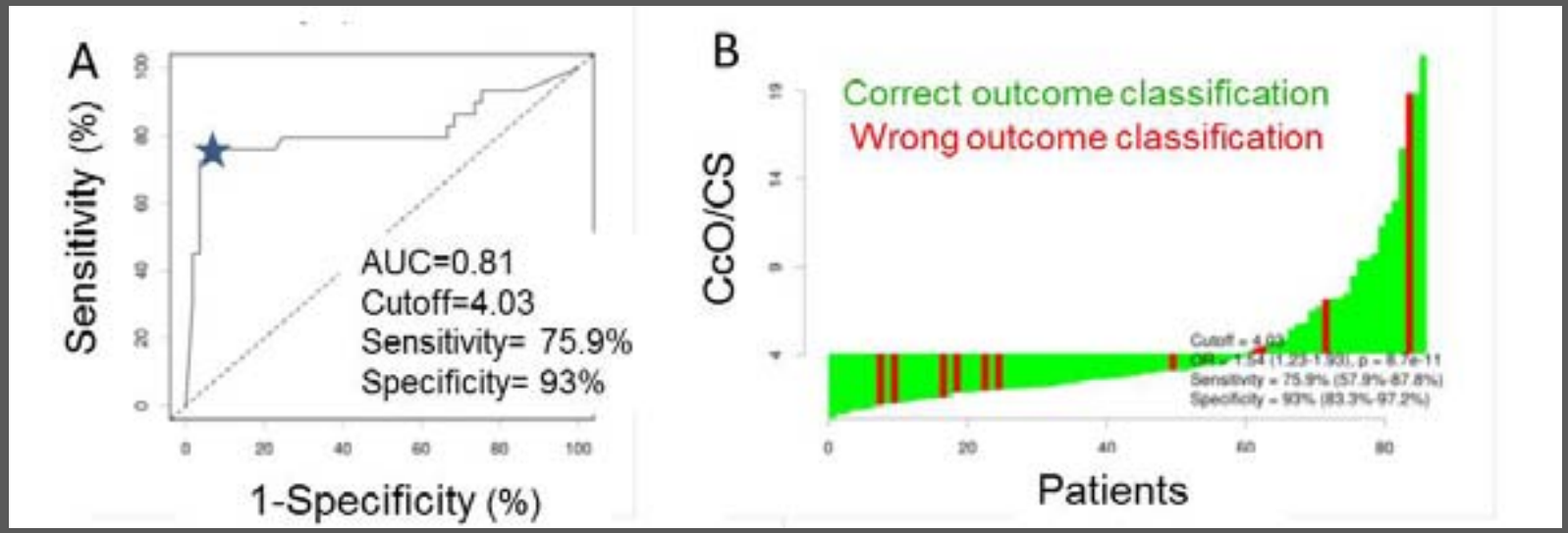
ROC for CcO Biomarker



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Comparison MGMT versus CcO in retrospective setting

Table 1: Performance of MGMT promoter methylation status and CcO activity as risk prediction models

Methyl-MGMT (good prognostic)	UnMethyl-MGMT (poor prognostic)	Low CcO (good prognostic)	High CcO (poor prognostic)
OS = 22 months	OS = 12 months	OS =14- 20 months	OS = 6.3, 6.5 months
Hegi et al 2005	Hegi et al. 2005	Griguer, et al., 2013	Griguer, et al., 2013
1 Year survival = 70%	1 Year survival = 52%	1 Year survival = 61%	1 Year survival = 0%

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Why a prospective biomarker trial?

- First trial was performed in a retrospective manner and consisted of relatively small numbers of patients.
- Patients had been adjunctively treated using either pre-Stupp or post-Stupp regimens.
- In a prospective trial, all patients will receive the post-Stupp standard of care therapy
- Since ~30% of patients have high CcO activity (poor risk) at diagnosis, it is important to control for the uneven distribution
- If the prospective biomarker study is confirmatory, it will likely have a major impact on how subsequent interventional trials will be conducted.
- Defining CcO activity will intensify development of targeted therapeutics.

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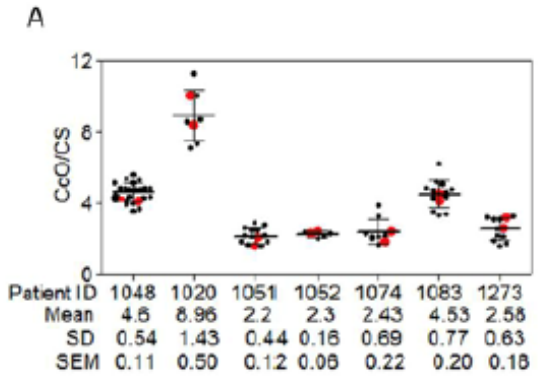
Reproducibility of CcO assay



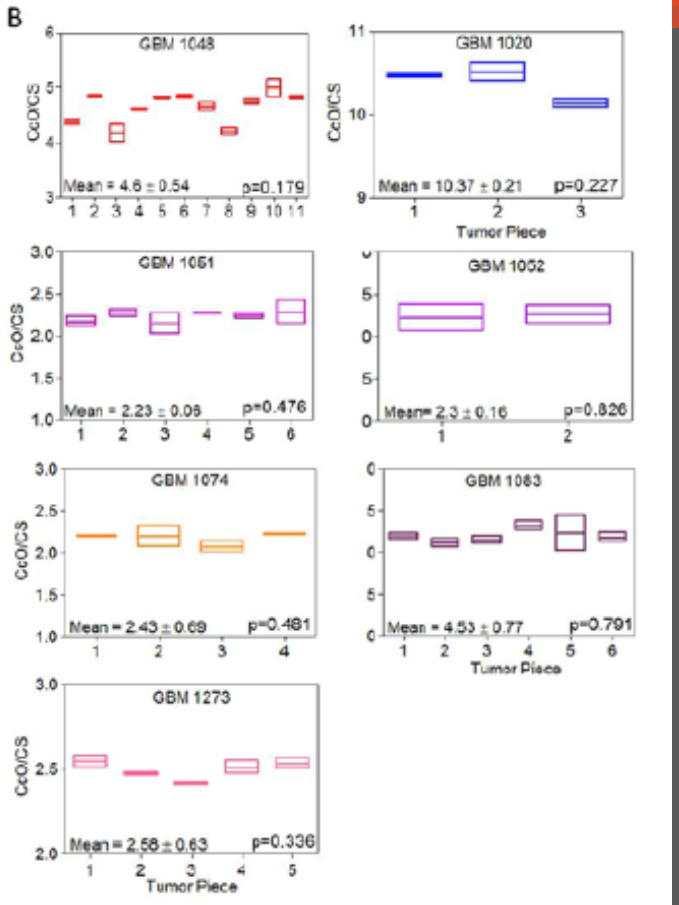


Figure 5: Intra-tumor distribution of CcO/Cs value. **(A)** CcO/CS ratio from different pieces of the same tumor (Mean ± SD). Each black dot represents the CcO/CS value from a different piece of the same tumor. Red dots represent the CcO/CS value determined for the same tumor during our retrospective clinical trial (25). **(B)** Bar in each graphs shows the CcO/CS ratio from different pieces of the same tumors. Bars represent the results of at least two independent determinations ± SEM. P value from test of within-subjects effects, repeated measures ANOVA.

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Intra-tumor Heterogeneity of CcO Activity

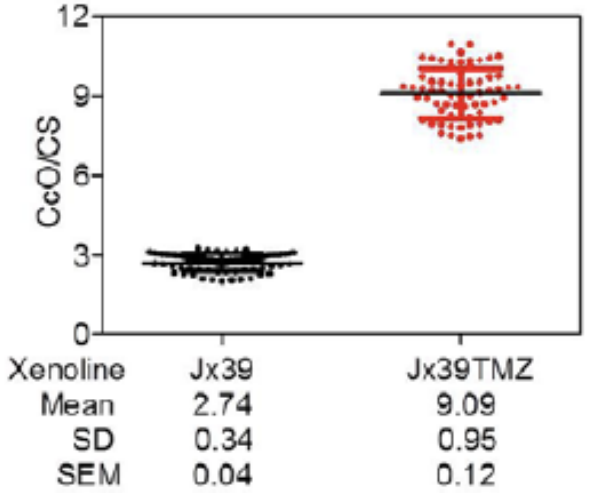

Figure 6: Intra- and inter-tumor distribution of CcO/Cs value. Ten whole tumors from Jx39, a xenoline with low CcO/CS ratio, and ten whole tumors from Jx39TMZ, a xenoline with high CcO/CS ratio. The tumors were collected from mice between 09/2013 and 02/2015 and divided in approximately 50-mg pieces prior to analysis (Jx39, 35 pieces; Jx39TMZ, 32 pieces).

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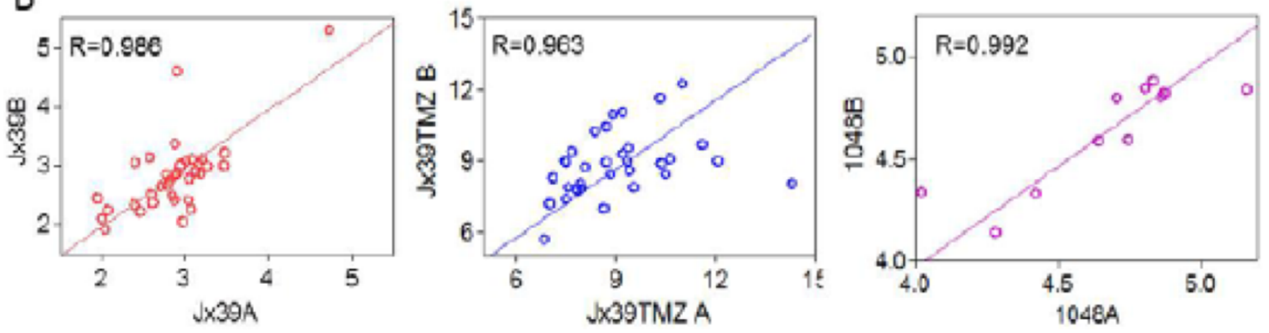
Accuracy and precision of CcO assay

Figure 7: Summary of statistical analysis to assess the precision and accuracy of CcO activity assay **(A)**; Scatter diagram of Spearman correlation with line of equality **(B)**.

A

Sample	n	Wilcoxon test p value	Spearman's coefficient	P value	Reproducibility value
Jx39	35	0.5467	0.567	0.0004	0.0002
Jx39TMZ	32	0.5440	0.463	0.0076	0.0075
GBM 1048	11	0.8710	0.827	0.0017	0.0051

B



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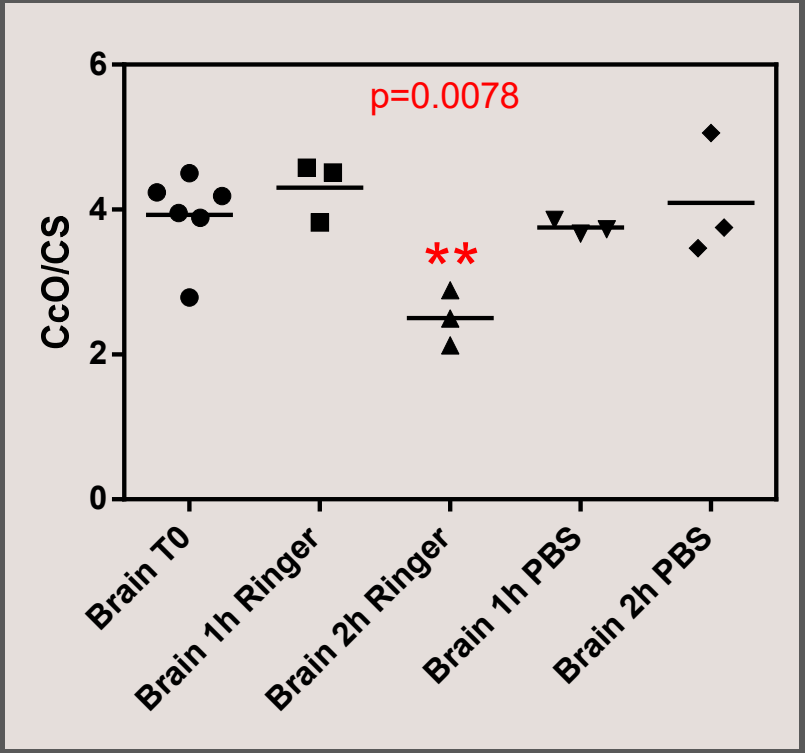
Effect of incubation time/buffer on CcO activity from normal brain tissue



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The NeuroNEXT Network

- Clinical Coordinating Center (CCC) at MGH
- Data Coordinating Center (DCC) at the University of Iowa
- Clinical Study Sites (CSS) at 25 academic institutions
- Central IRB (CIRB)
- Master Clinical Trial Agreements (MCTA) with all CSS

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NN- 106

Prospective Study of Cytochrome C Oxidase Activity as a Novel Biomarker In Subjects With Newly Diagnosed Primary Glioblastoma Multiforme

NINDS-U01NS093663

NN-106 TEAM

UAB Component:	
• James Markert	Role: Neurosurgeon Co.Pi
• Melanie Benge	Role: Clinical Coordinator
• Burt Nabors	Role: Disease Specialist
• James Hackney	Role: Central Pathology
• Robbie Minor	Role: Patient Advocate (ABTA)
• Roger Packer	Role: Disease Specialist (CNMC)
DCC Component:	
• Christopher Coffey	Role: DCC Lead Director
• Dixie Ecklund	Role: Associate Director
• Michael Bosch	Role: NN DCC Coordinator
• Eric Foster	Role: NN DCC lead Biostatistician
CCC Component:	
• Merit Cudkowicz	Role: CCC Lead Director
• Brenda Thornell	Role: CCC Project Manager
• Marianne Kearney Operations	Role: CCC Director of Research
• Katy Mahoney	Role: Project Manager
• Melisa Ricker	Role: Ass. Project Manager
UI- Rad-Onc.:	
• Corinne E Griguer	Role: Contact PI
• Claudia Oliva	Role: Assays specialist
• Kelly Bodeker	Role: Regulatory specialist

NN-106 Clinical Sites

- UAB
- UTSW
- Utah
- CUMC
- Rochester
- Cincinnati
- Northwestern
- Miami
- OHSU
- UPMC
- Einstein Montefiore
- Suny SB
- Swedish HS
- Wash U
- OSU
- UC Davis
- Kansas
- Vanderbilt
- MGH

Overall Objective

- To define CcO activity as a novel prognostic biomarker for GBM patients receiving standard of care therapy

Primary Objective

- To demonstrate that **overall survival (OS) inversely correlates with Cytochrome c Oxidase (CcO) activity in newly diagnosed primary GBM tumors, treated by standard of care**

Secondary Objectives

- To study the relation between CcO activity and progression free survival times (PFS; time intervals from dates of diagnosis to documented disease progression by MRI or tumor-related death)
- To compare the prognostic abilities of CcO activity to other frequently used biomarkers, namely the methylation status of O6-methylguanine–DNA methyltransferase (MGMT) on OS and PFS

Inclusion Criteria: *Subject Characteristics*

- Willingness to undergo a maximal debulking surgery and radiotherapy concomitant with temozolomide at initial diagnosis
- Willingness and ability to provide written informed consent and to comply with the study protocol as judged by the investigator.
- Age ≥ 21 years.
- KPS score ≥60
- No history of other malignancies except adequately treated non-melanoma skin cancer, curatively treated *in situ* cancer of the cervix, or other curatively treated solid tumors with no evidence of disease for at least 5 years
- No serious active infection (e.g., wound infection requiring parenteral antibiotics) or other serious underlying medical conditions that would preclude study treatment
- No other condition (e.g., psychological or geographical) that would preclude study compliance

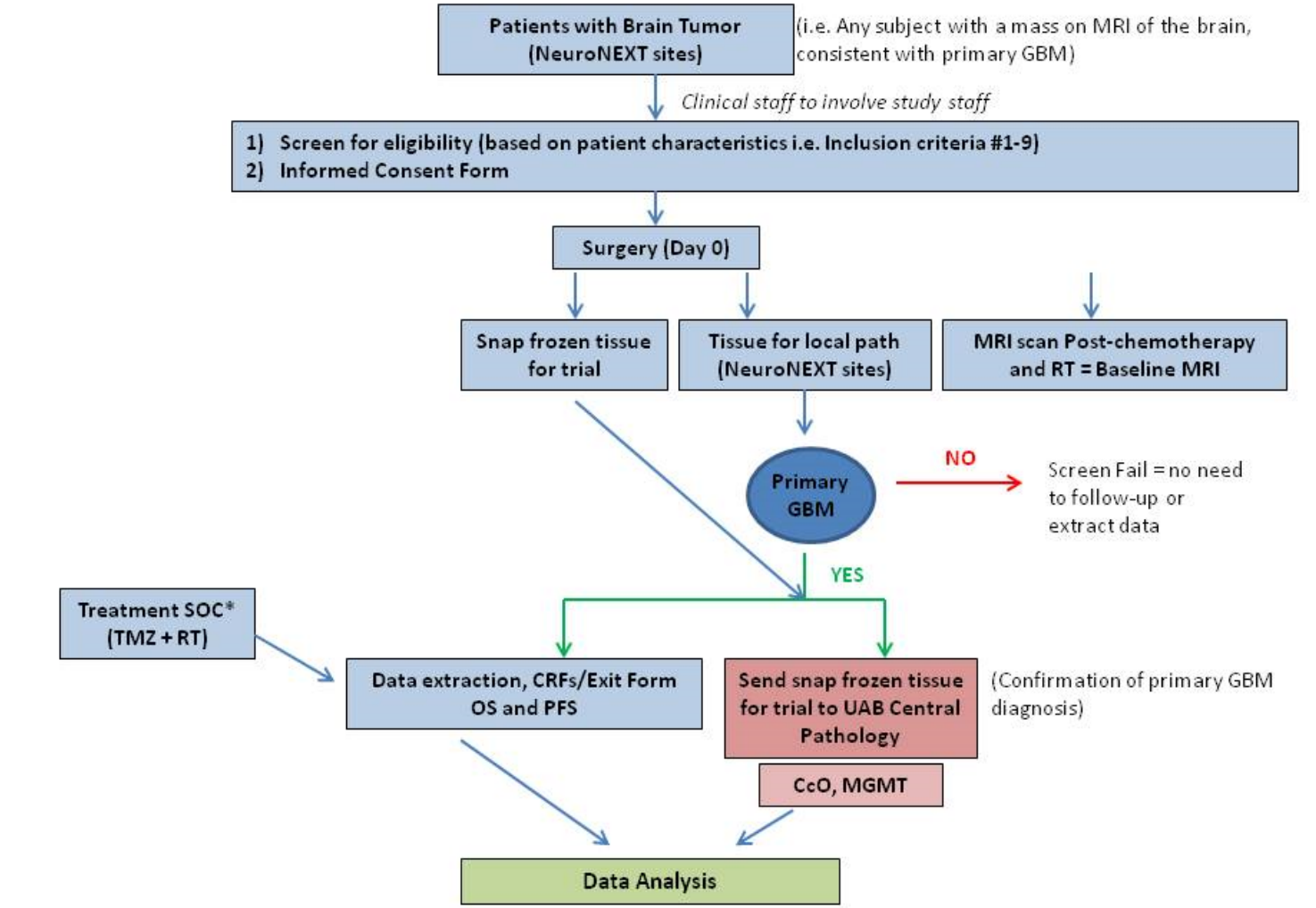
Inclusion Criteria: *Disease Characteristics*

- Histologically confirmed newly diagnosed Primary GBM before treatment using WHO classification criteria
- Viability of tumor tissue representative of GBM ≥ 70 mg, snap-frozen within 30 minutes of resection [no more than 10 minutes on ice (4°C)]
- All subjects must have received safe gross resection followed by standard radiation with concomitant Temozolomide taken during the course of radiation therapy

Exclusion Criteria

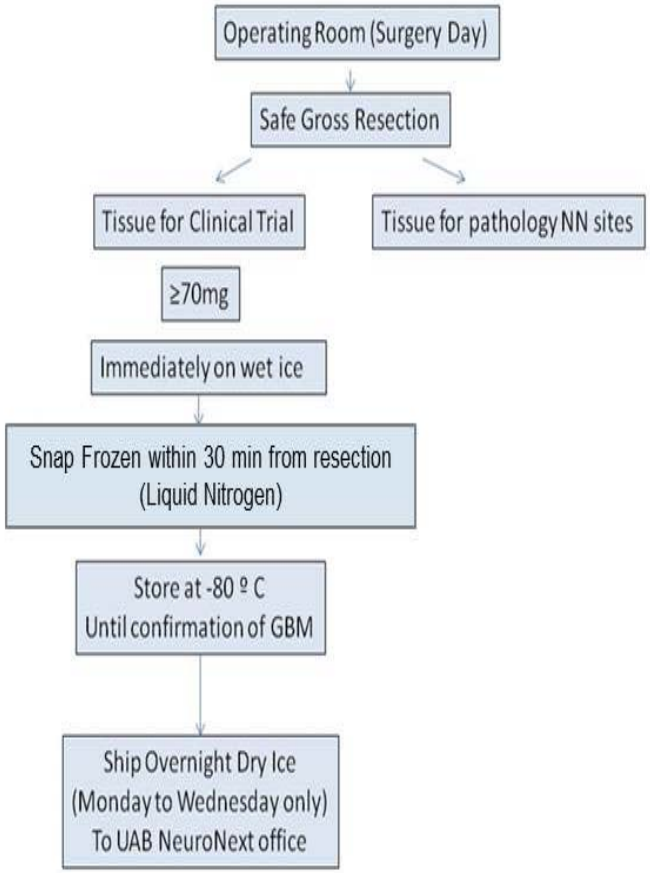
- Inability to fulfill the requirements of the protocol
- Any serious disease or condition that, in the opinion of the investigator, would compromise the subject's ability to participate in the study.
- Secondary GBM or other gliomas.
- History of sensitivity to Temodar.
- Planned upfront treatment with any anti-angiogenic agent targeting the VEGF pathway including but not limited to bevacizumab, cediranib, vandetanib, sunitinib,pazopanib, aflibercept, or sorafenib or any immunotherapy regimen.
- GLIADEL wafers in combination with surgical resection.

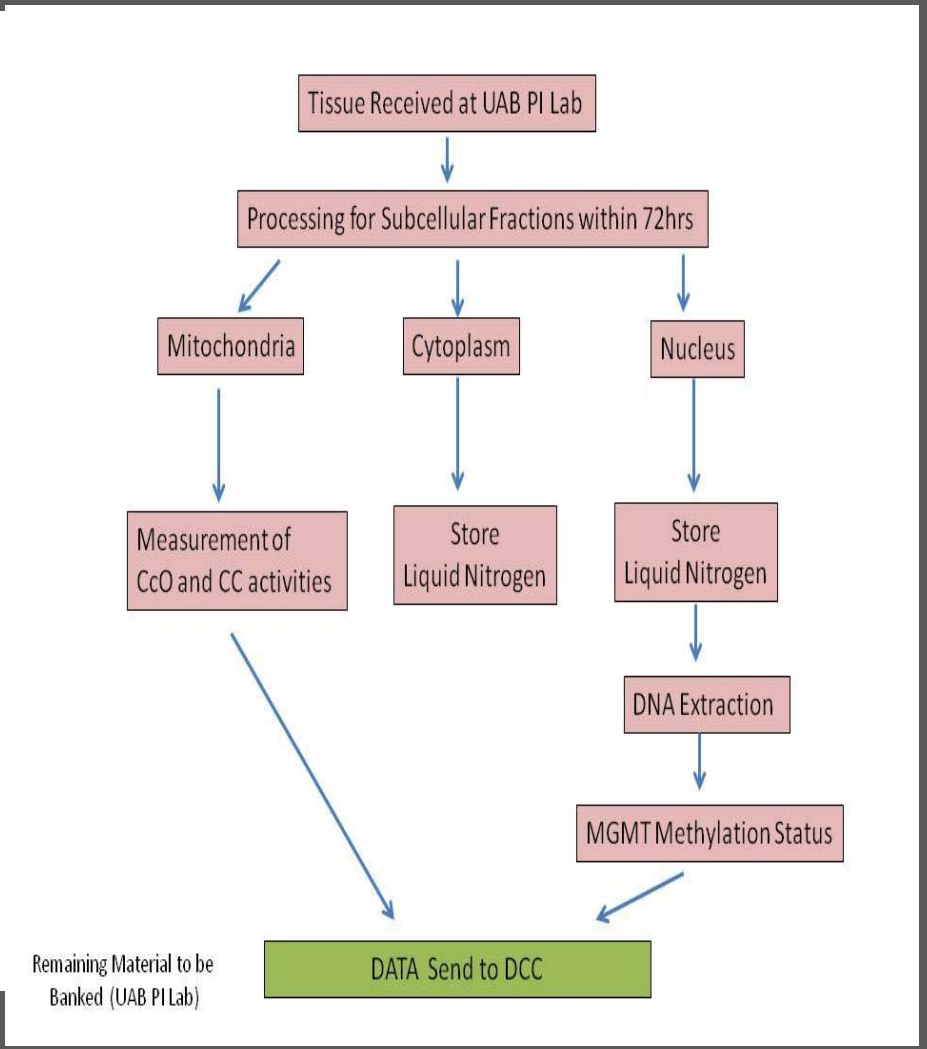
Trial Design Roadmap

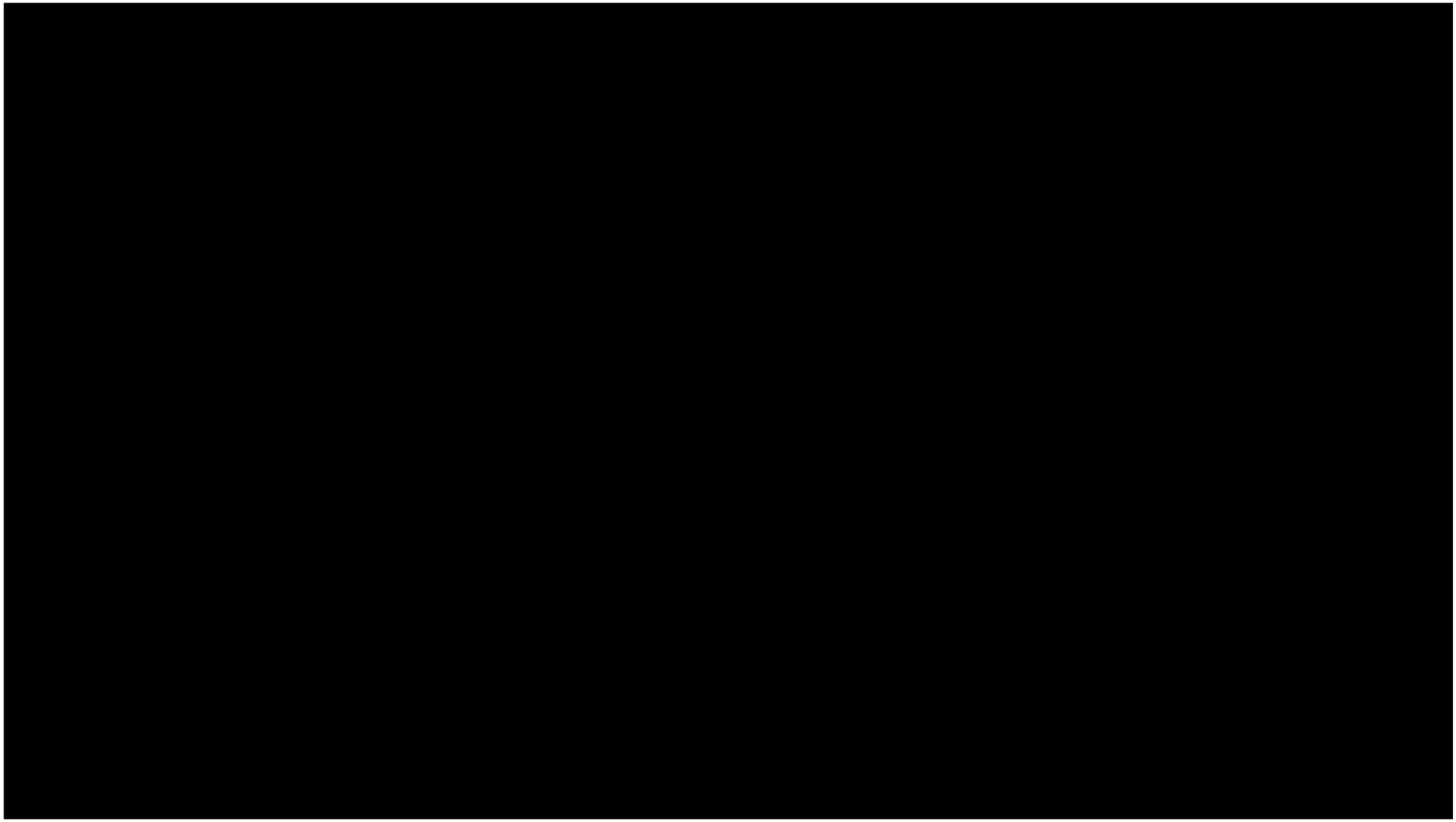


*Can include Optune™ or any other treatment with Temozolomide (TMZ) and Radiation therapy (RT), with the exception of Avastin and any immunotherapy regimen

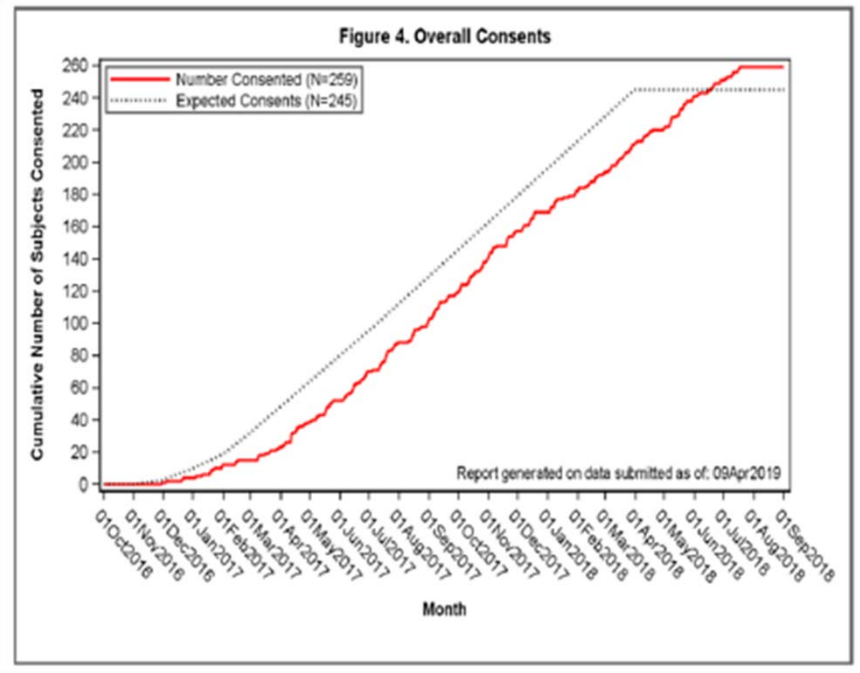
Laboratory Data Flow

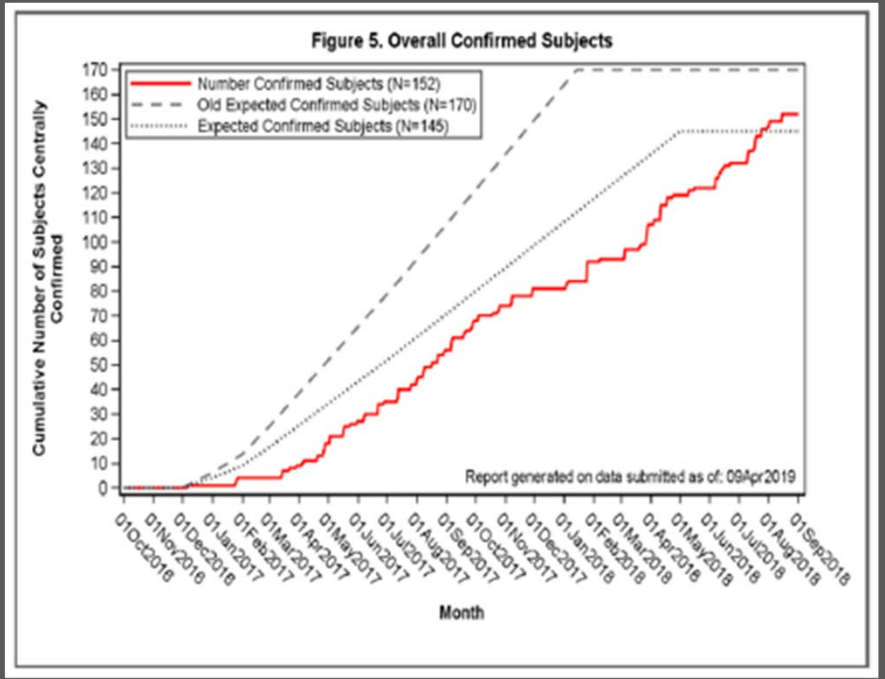






Updates





- Consented subjects: 259
- Eligible subjects (sites): 154
- Eligible (central): 152
- Ineligible subjects: 104

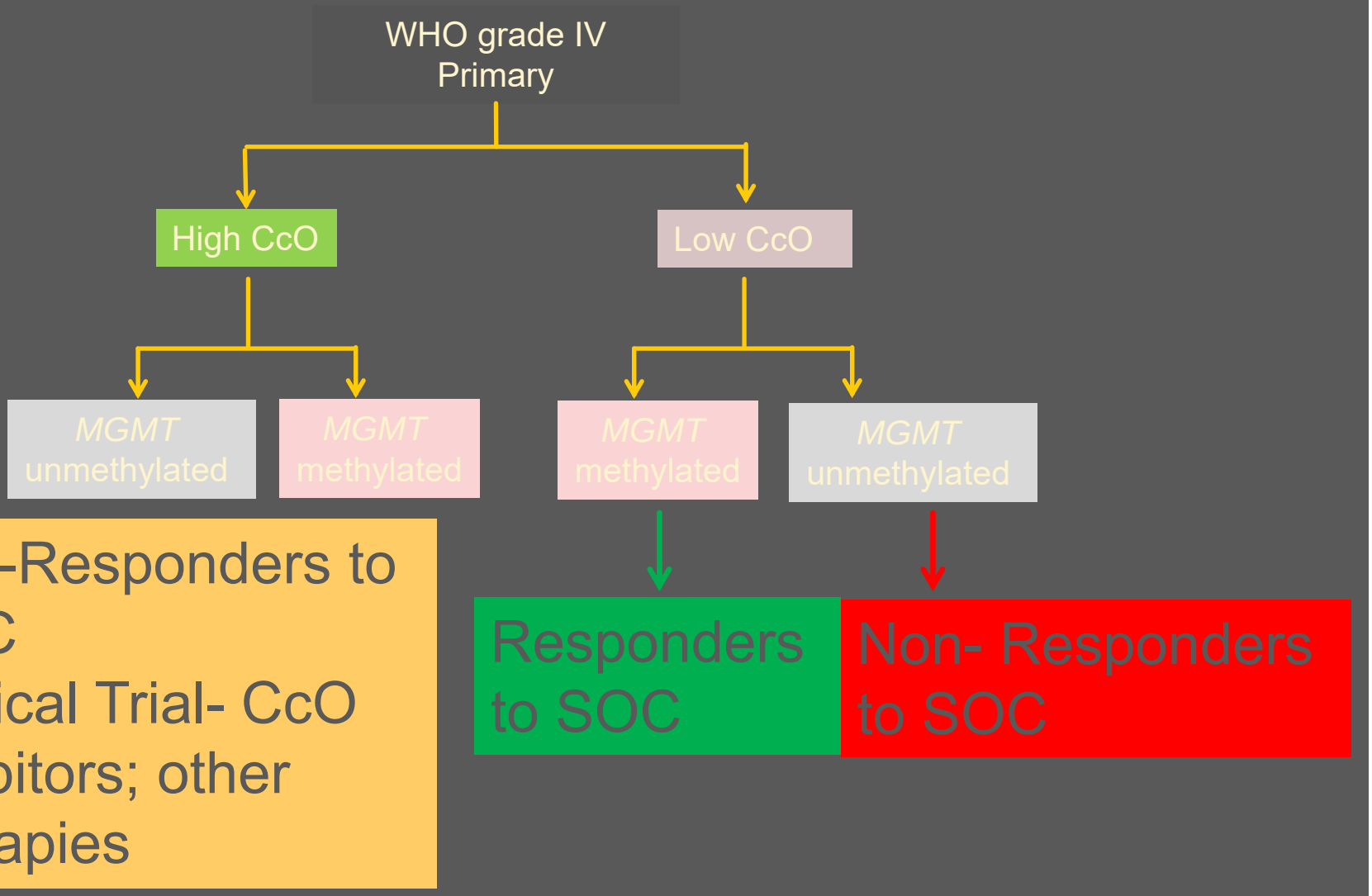
- Death : 84
- 24 Month Extraction: 8
- Early Termination: 1

Next Steps

- Definition of subpopulation of GBM with high CcO (short term survival) will facilitate the design of future clinical trial for testing new drugs against GBM. For example, it is possible that a drug may show benefit in one subgroup but not the other.

- Selective inhibitors of CcO are in pre-clinical development. Envision to be beneficial for the High CcO/low OS GBM patient population.

Biomarker-Driven Treatment



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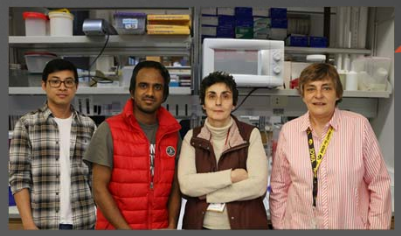

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