

EFFECT OF P-bi-TAT TREATMENT ON EXPRESSION OF HUMAN CANCER SURVIVAL GENES

Human cancer survival predictor genes are genes expression of which is highly significantly associated with long-term clinical outcomes in cancer patients such as 5-year survival and cancer-specific death. This highly relevant clinically category of genes was identified in a recent study of global gene expression patterns of all protein-coding genes in the clinical samples of 33 different human cancers from 9,666 patients of The Cancer Genome Atlas (TCGA) and the gene expression patterns in 37 normal human tissues obtained from 162 healthy subjects in the Human Protein Atlas (HPA) project (Uhlen et al., Science 357, 660; 2017; published on August 18, 2017). In total, more than 100 million Kaplan-Meier plots were generated that corresponded to all 19,571 protein-coding genes across the 17 cancer types. Ultimately, these experiments identified prognostic genes for 17 different human cancer types. Prognostic genes were defined as genes for which the expression level above or below the experimentally determined cutoff in an individual patient yields a significant ($P < 0.001$) difference in patient's survival.

Genome-wide gene expression profiling experiments revealed that human cancer survival genes are markedly enriched among genes expression of which is significantly altered in human cancer cells treated with P-bi-TAT. P-bi-TAT therapy significantly alters the expression of thousands cancer survival genes.

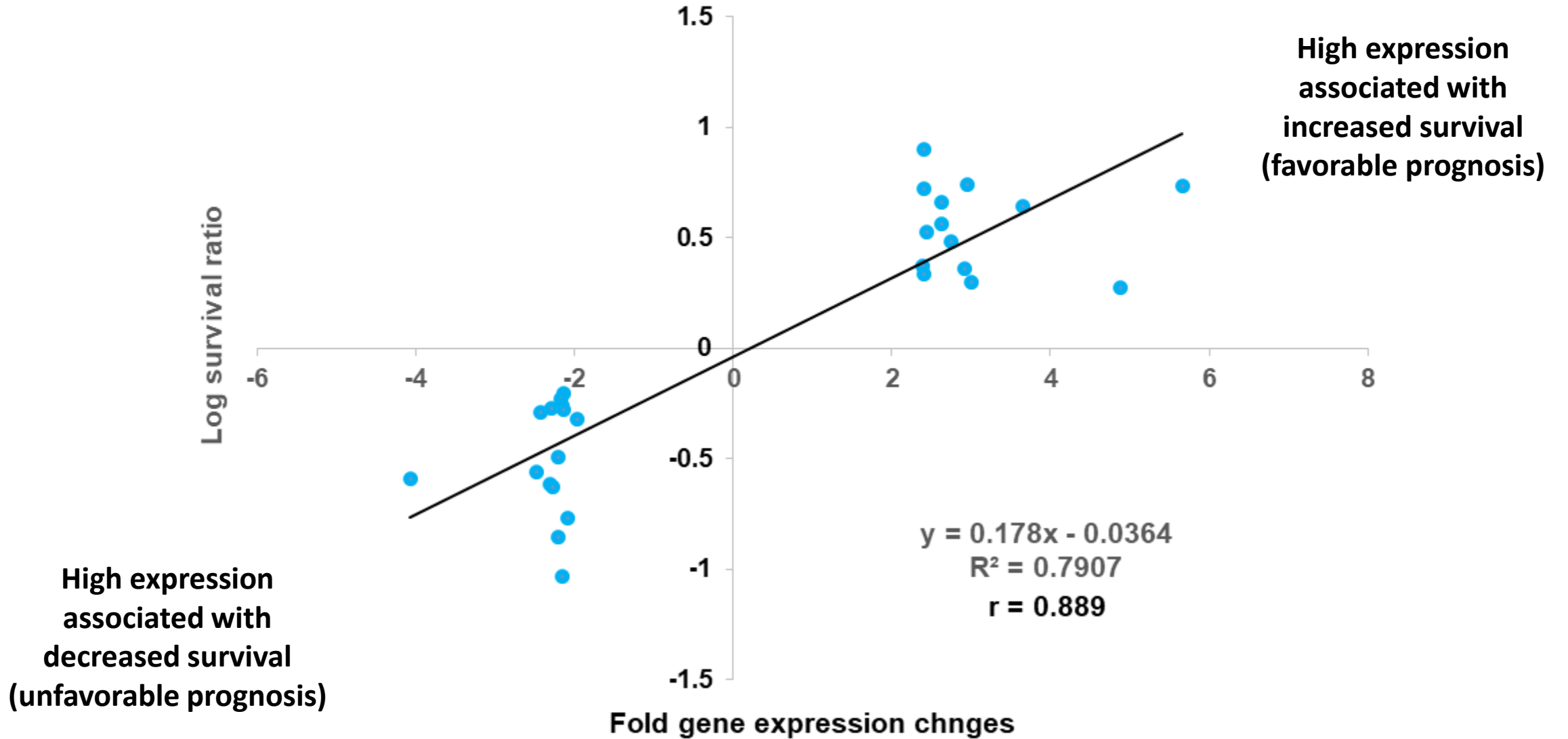
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There are two categories of human cancer survival predictor genes:

- 1) Genes for which increased expression is significantly associated with favorable prognosis (increased likelihood of survival and decreased probability of death from cancer: longer patients survival defined as good outcome);**
- 2) Genes for which increased expression is significantly associated with unfavorable prognosis (decreased likelihood of survival and increased probability of death from cancer: shorter patients survival defined as poor outcome);**

Our experiments identified gene expression signatures reflecting a transcriptional reversal of death from cancer phenotypes induced by the P-bi-TAT therapy: increased expression of genes associated with favorable clinical outcomes and decreased expression of genes associated with unfavorable clinical outcomes.

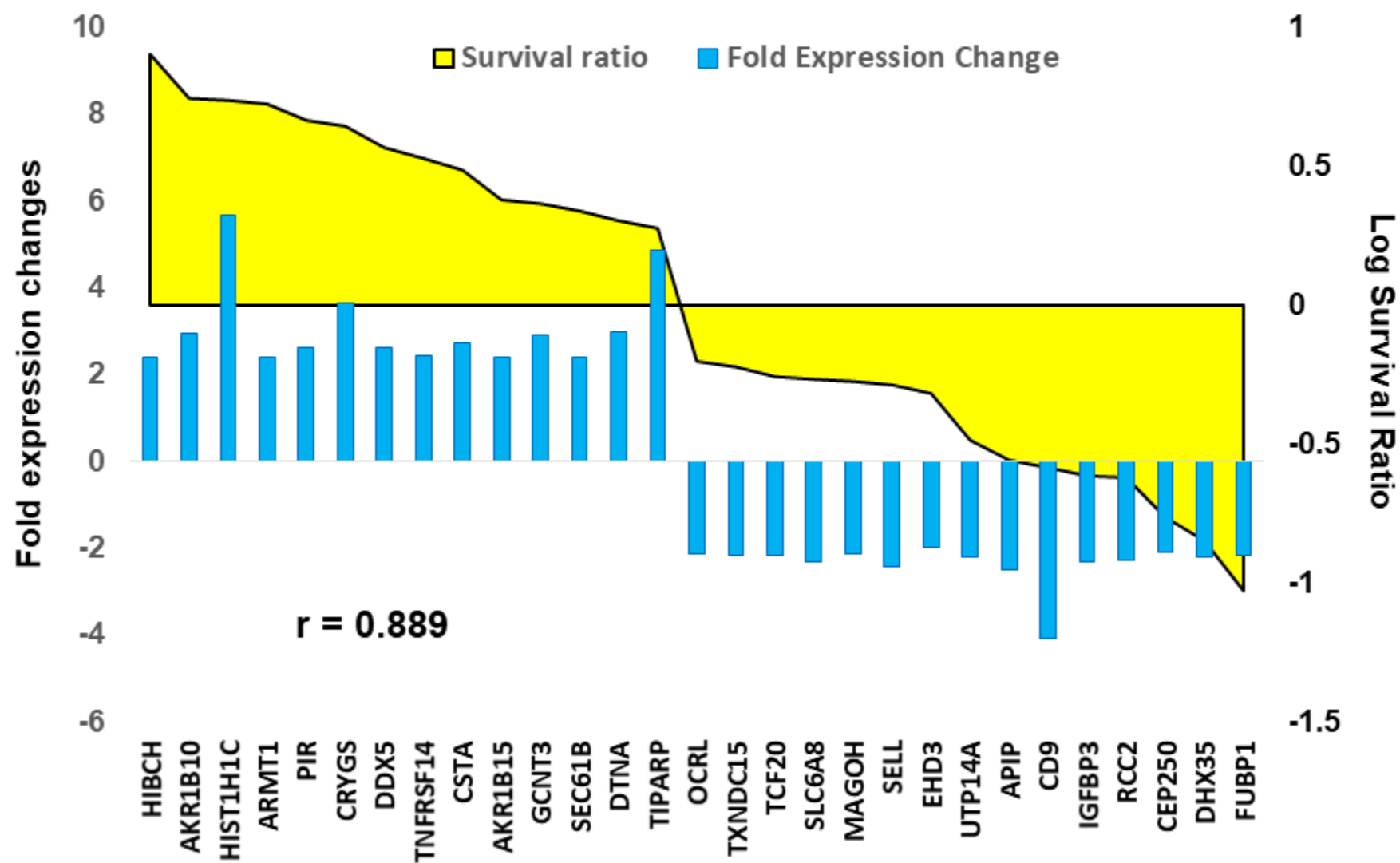
Correlation between the positive and negative effects of P-bi-TAT treatment on expression of genes associated with increased or decreased likelihood of cancer patients' survival



Effect of P-bi-TAT therapy on cancer survival predictor genes: increased expression of genes associated with favorable prognosis and decreased expression of genes associated with unfavorable clinical outcome

| Gene Symbol | Fold Expression Change | P-value | Log Survival Ratio | Prognosis (cancer types) |
|-------------|------------------------|---------|--------------------|---|
| HIBCH | 2.39 | 0.018 | 0.902483 | favorable (renal; ovarian) |
| AKR1B10 | 2.95 | 0.0135 | 0.74366 | favorable (colorectal) |
| HIST1H1C | 5.66 | 0.0089 | 0.736966 | favorable (urothelial) |
| ARMT1 | 2.39 | 0.0242 | 0.723482 | favorable (colorectal) |
| PIR | 2.61 | 0.025 | 0.664899 | favorable (cervical; ovarian) |
| CRYGS | 3.64 | 0.0048 | 0.643856 | favorable (urothelial) |
| DDX5 | 2.62 | 0.0017 | 0.566347 | favorable (colorectal) |
| TNFRSF14 | 2.43 | 0.0108 | 0.527423 | favorable (urothelial; endometrial; breast) |
| CSTA | 2.73 | 0.0119 | 0.485427 | favorable (head & neck) |
| AKR1B15 | 2.38 | 0.0032 | 0.378512 | favorable (head & neck) |
| GCNT3 | 2.9 | 0.0039 | 0.364997 | favorable (endometrial) |
| SEC61B | 2.39 | 0.009 | 0.338802 | favorable (endometrial) |
| DTNA | 3 | 0.0002 | 0.303781 | favorable (renal) |
| TIPARP | 4.87 | 0.0385 | 0.276518 | favorable (breast) |
| OCRL | -2.14 | 0.0033 | -0.20249 | unfavorable (breast) |
| TXNDC15 | -2.18 | 0.0171 | -0.22239 | unfavorable (renal) |
| TCF20 | -2.17 | 0.0047 | -0.25716 | unfavorable (liver) |
| SLC6A8 | -2.3 | 0.0318 | -0.26685 | unfavorable (renal; thyroid) |
| MAGOH | -2.14 | 0.0226 | -0.27462 | unfavorable (renal) |
| SELL | -2.44 | 0.0279 | -0.28728 | unfavorable (renal) |
| EHD3 | -1.97 | 0.0467 | -0.31748 | unfavorable (renal) |
| UTP14A | -2.22 | 0.0275 | -0.48625 | unfavorable (head & neck; renal) |
| APIP | -2.49 | 0.0092 | -0.558 | unfavorable (liver) |
| CD9 | -4.08 | 0.0085 | -0.58496 | unfavorable (pancreatic) |
| IGFBP3 | -2.32 | 0.0253 | -0.61444 | unfavorable (colorectal; liver; renal) |
| RCC2 | -2.28 | 0.0288 | -0.62105 | unfavorable (liver; renal; melanoma) |
| CEP250 | -2.1 | 0.0263 | -0.76332 | unfavorable (liver; renal) |
| DHX35 | -2.22 | 0.0008 | -0.84812 | unfavorable (liver; endometrial) |
| FUBP1 | -2.16 | 0.0274 | -1.02748 | unfavorable (liver) |

Effect of P-bi-TAT therapy on cancer survival predictor genes:
increased expression of genes associated with favorable prognosis
and decreased expression of genes associated with unfavorable
clinical outcome

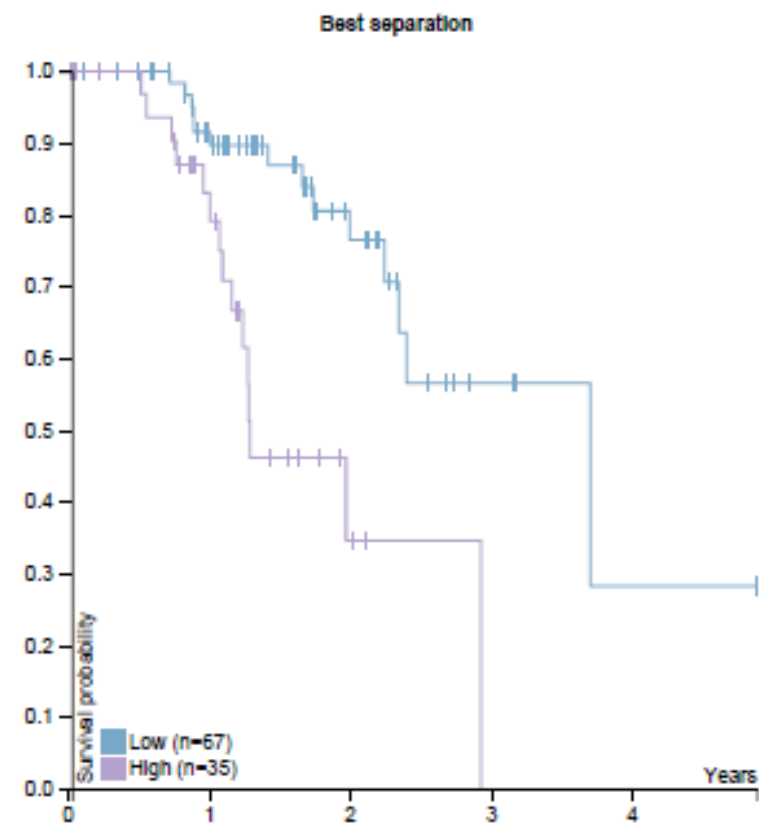


Survival and expression agreements

Melanoma

UNG

MELANOMA - Survival analysis[†]



Expression cutoff[†]

20.0 FPKM

3-year survival high[†]

0%

3-year survival low[†]

56%

Log-rank P value[†]

3.06e-4 (Prognostic, unfavourable)

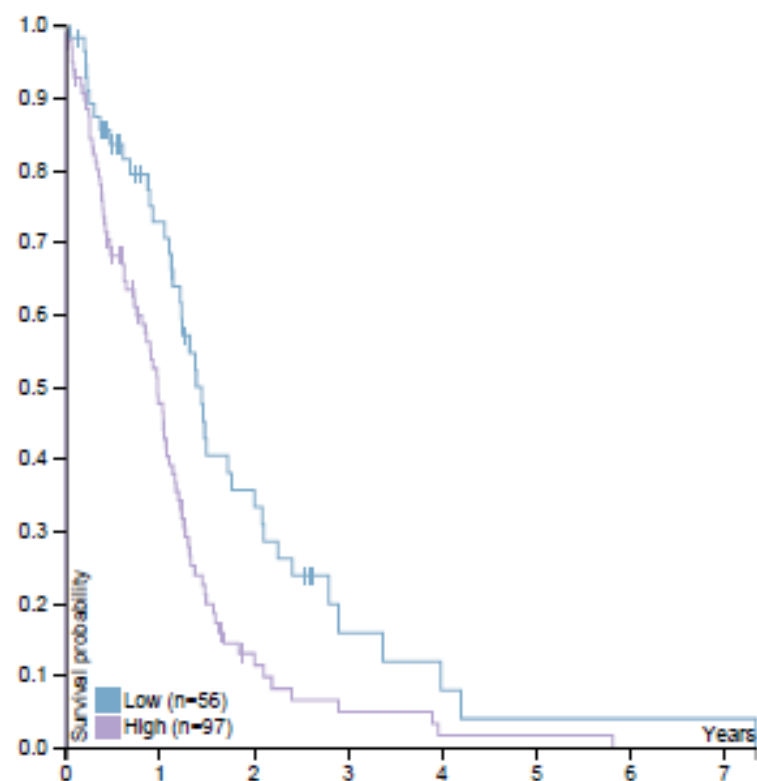
Survival and expression agreements

Glioma

SDC1

GLIOMA - Survival analysis¹

Best separation



Expression cutoff¹

3.3 FPKM

3-year survival high¹

3%

3-year survival low¹

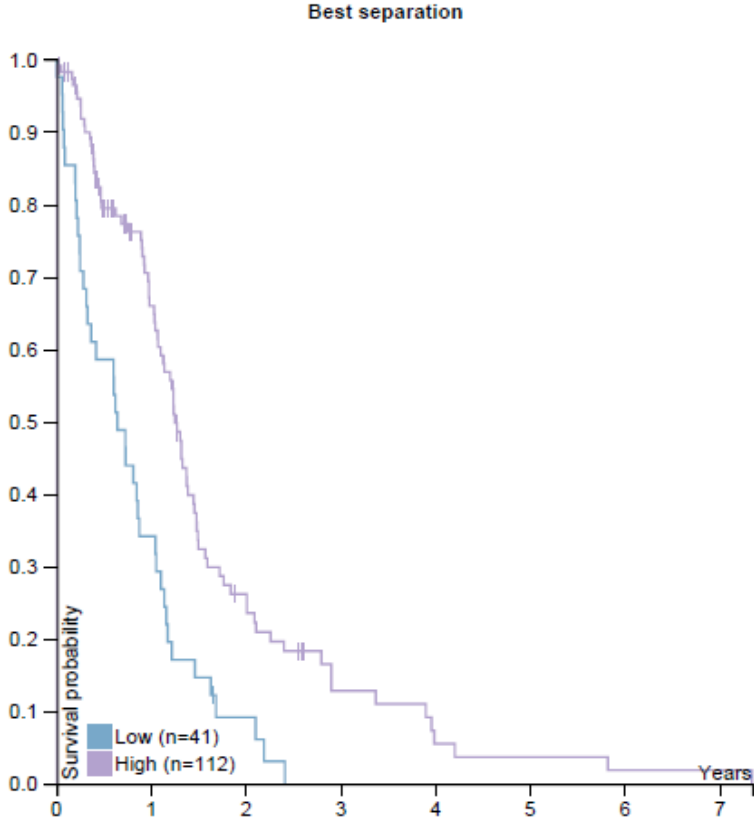
12%

Log-rank P value¹

8.05e-4 (Prognostic, unfavourable)

ZBTB6

GLIOMA - Survival analysisⁱ



Expression cutoffⁱ

3.6 FPKM

3-year survival highⁱ

11%

3-year survival lowⁱ

0%

Log-rank P valueⁱ

9.75e-6 (Prognostic, favourable)

Up-regulated GBM

Examples from consensus
signature

CONSENSUS SET OF 737 GENES AFFECTED BY P-bi-TAT THERAPY

Primary human glioblastoma cells

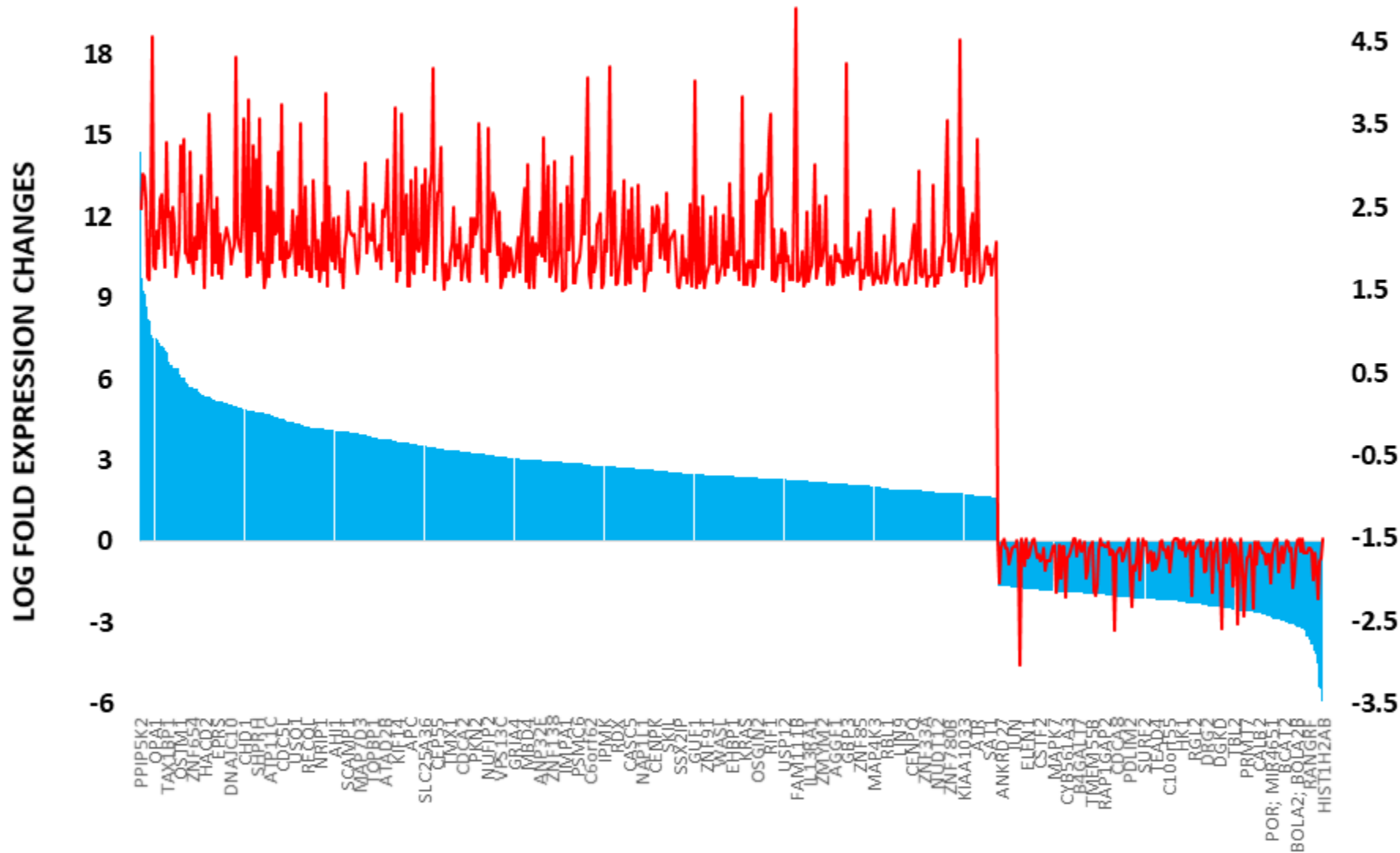
GBM 021913

Human metastatic pancreatic cancer cell line

SUIT2-luc

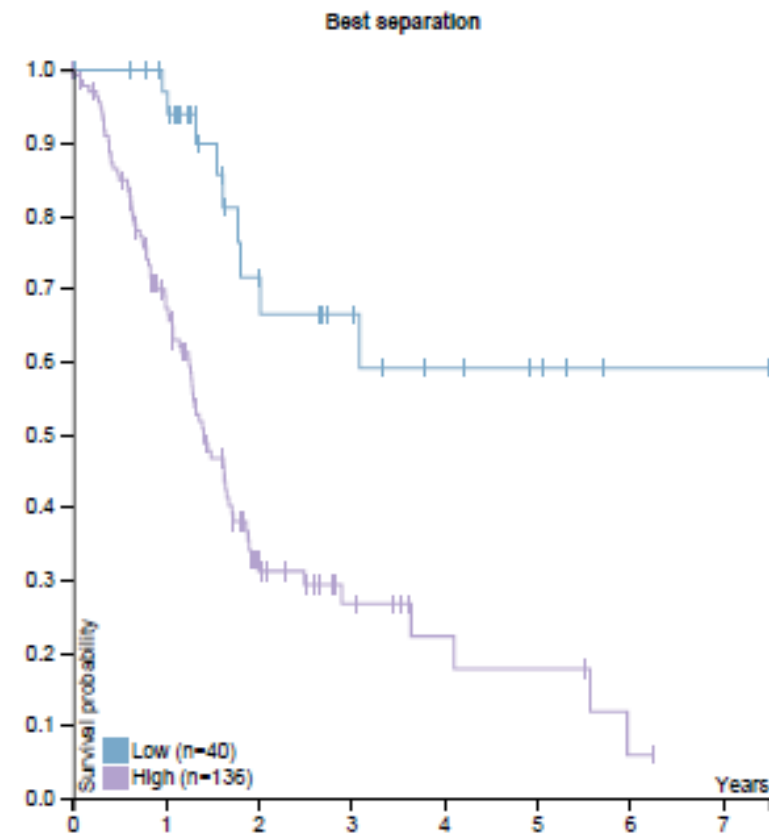
CONSENSUS SET OF 737 GENES AFFECTED BY P-bi-TAT THERAPY

■ GBM P-bi-TAT — SUI2 P-bi-TAT



ZNF185

PANCREATIC CANCER - Survival analysis¹



Expression cutoff¹

4.7 FPKM

5-year survival high¹

18%

5-year survival low¹

59%

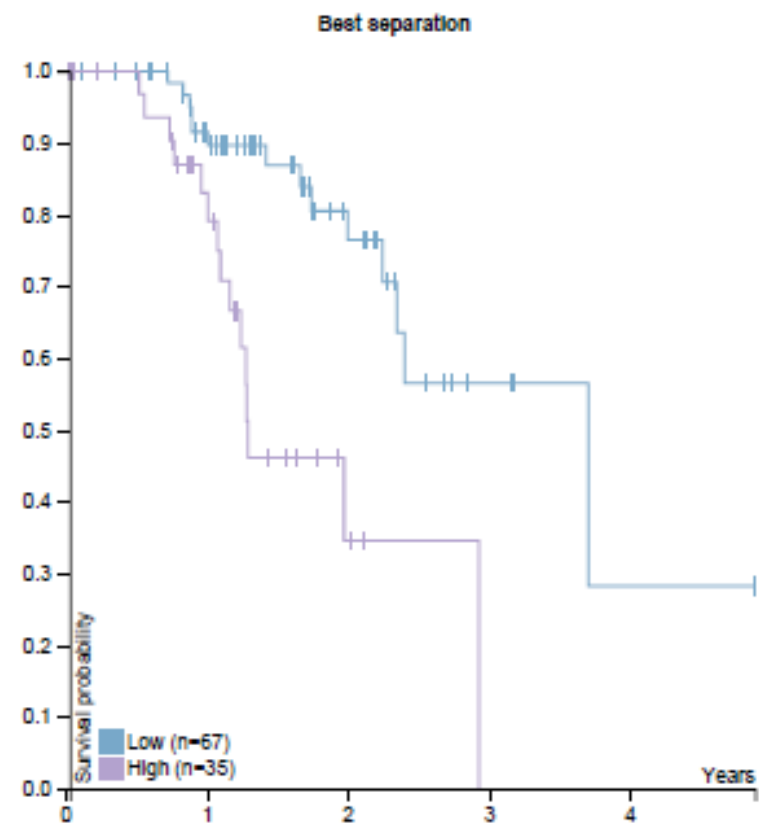
Log-rank P value¹

3.30e-5 (Prognostic, unfavourable)

Down-regulated

UNG

MELANOMA - Survival analysis[†]



Expression cutoff[†]

20.0 FPKM

3-year survival high[†]

0%

3-year survival low[†]

56%

Log-rank P value[†]

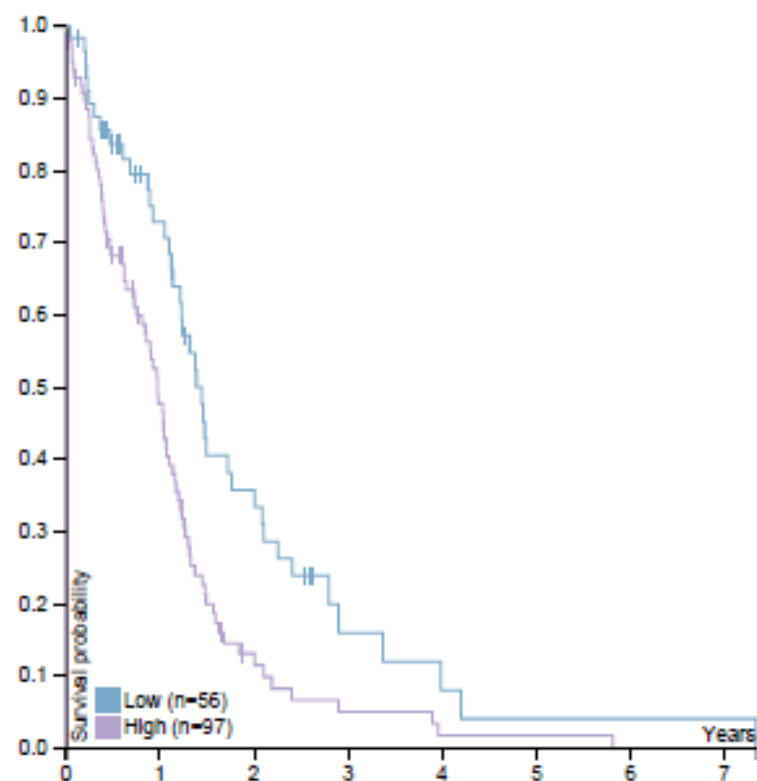
3.06e-4 (Prognostic, unfavourable)

Down-regulated

SDC1

GLIOMA - Survival analysis¹

Best separation



Expression cutoff¹

3.3 FPKM

3-year survival high¹

3%

3-year survival low¹

12%

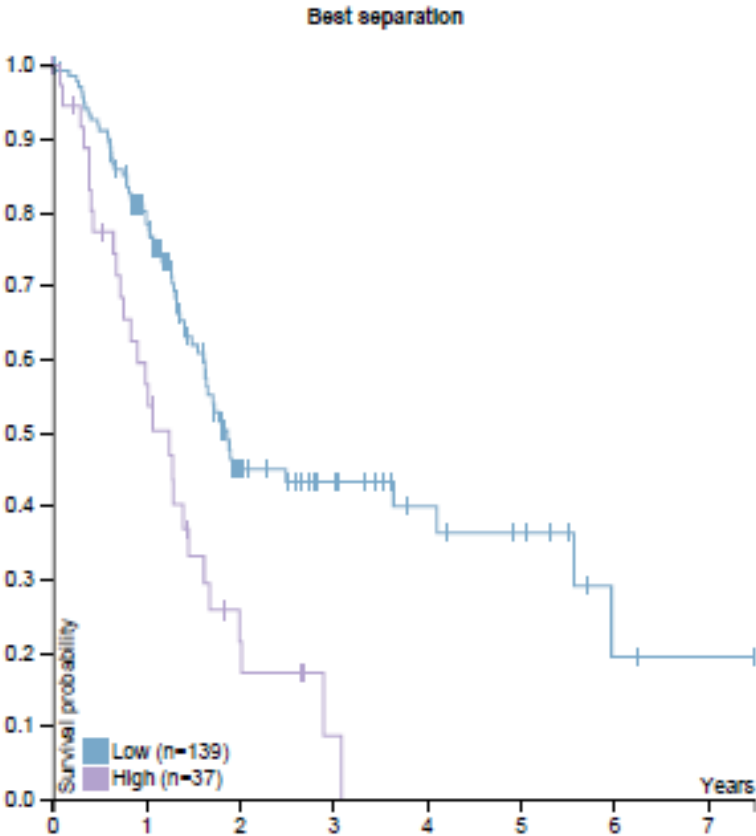
Log-rank P value¹

8.05e-4 (Prognostic, unfavourable)

Down-regulated

HIST1H1C

PANCREATIC CANCER - Survival analysis¹



Expression cutoff¹

108.2 FPKM

5-year survival high¹

0%

5-year survival low¹

36%

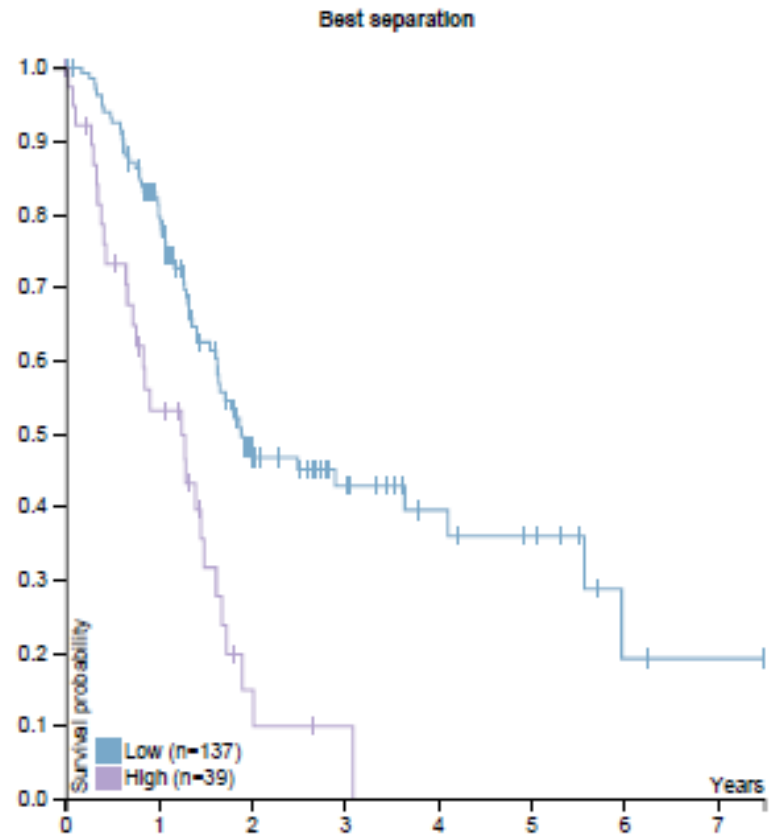
Log-rank P value¹

1.12e-4 (Prognostic, unfavourable)

Down-regulated (GBM)

HIST1H3H

PANCREATIC CANCER - Survival analysis¹



Expression cutoff¹

3.8 FPKM

5-year survival high¹

0%

5-year survival low¹

36%

Log-rank P value¹

9.25e-6 (Prognostic, unfavourable)

Down-regulated (GBM)