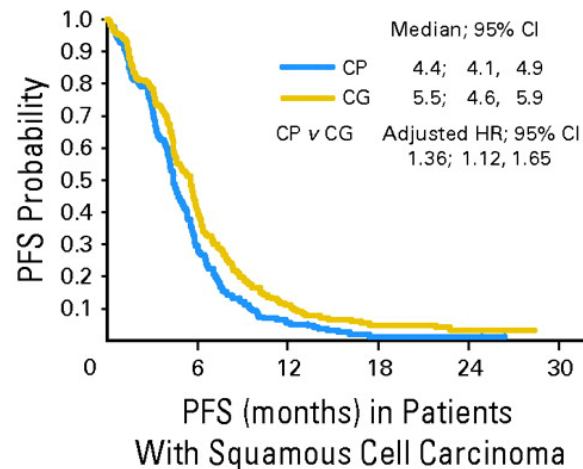
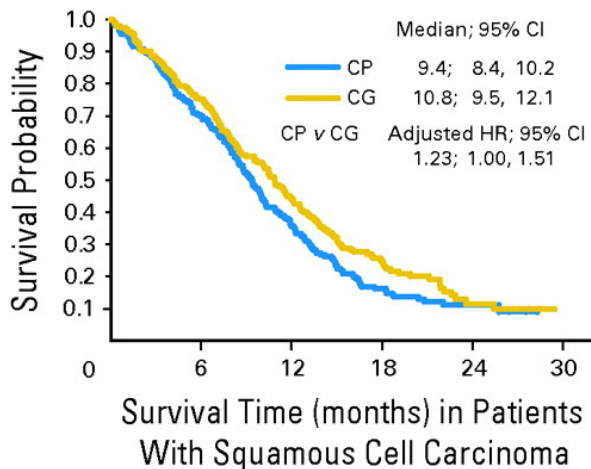
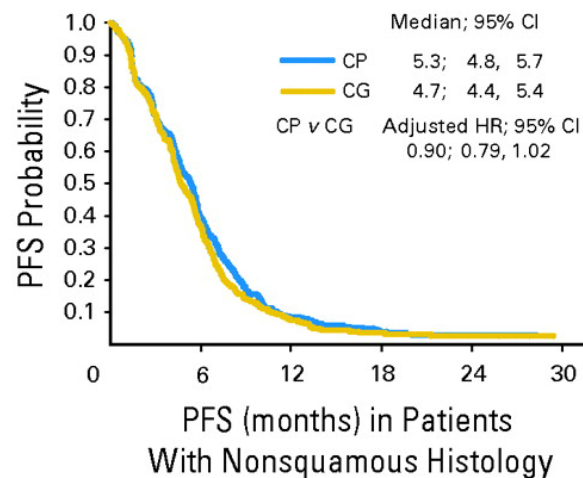
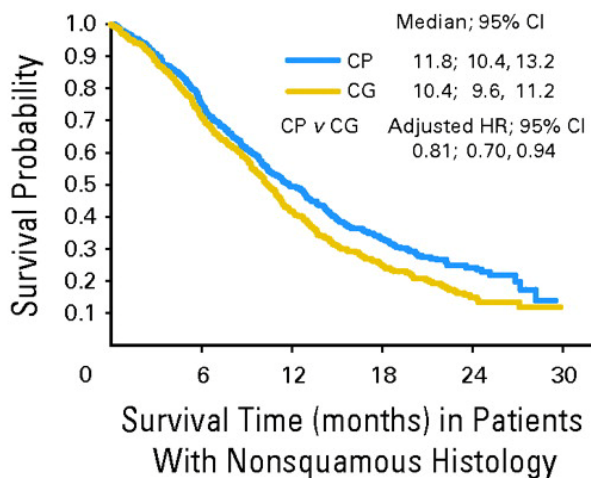


Thymidylate Synthase Expression and Sensitivity to Antifolates

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Cis/Pem vs. Cis/Gem in Advanced NSCLC



Efficacy by Histology in Pemetrexed Studies

NSCLC Histologic Group	Second-line Pem vs. Docetaxel		First-line Pem/Cis vs. Gem/Cis		Maintenance Pem vs. Placebo	
	Pem	Doc	Cis/Pem	Cis/Gem	Pem	Placebo
Non-squamous	n=205	n=194	n=618	n=634	n=325	n=156
Median OS, months	9.3	8.0	11.0	10.1	15.5	10.3
Adjusted HR (95% CI)	0.78 (0.61–1.00)		0.84 (0.74–0.96)		0.70 (0.56–0.88)	
P value	0.048		0.011		0.002	
Squamous	n=78	n=94	n=244	n=229	n=116	n=66
Median OS, months	6.2	7.4	9.4	10.8	9.9	10.8
Adjusted HR (95% CI)	1.56 (1.08–2.26)		1.23 (1.00–1.51)		1.07 (0.77–1.50)	
P value	0.018		0.050		0.678	

Non-squamous = adenocarcinoma, large cell carcinoma, and other/indeterminate NSCLC histology

Treatment-by-Histology Interactions

Efficacy Parameter	Second-line Pem vs Docetaxel		First-line Pem/Cis vs Gem/Cis		Maintenance Pem vs Placebo	
	Nsquam n=399	Squam n=172	Nsquam n=1252	Squam n=473	Nsquam n=482	Squam n=181
OS adjusted HR (95% CI)	0.78 (0.61, 1.00)	1.56 (1.08, 2.26)	0.84 (0.74, 0.96)	1.23 (1.00, 1.51)	0.66 (0.49, 0.88)	1.28 (0.85, 1.93)
THI - HR (95% CI) P value	0.48 (0.31, 0.74) 0.001		0.69 (0.54, 0.87) 0.002		0.52 (0.31, 0.86) 0.033	

THI=treatment-by-histology interaction

Scagliotti GV et al. JTO in press

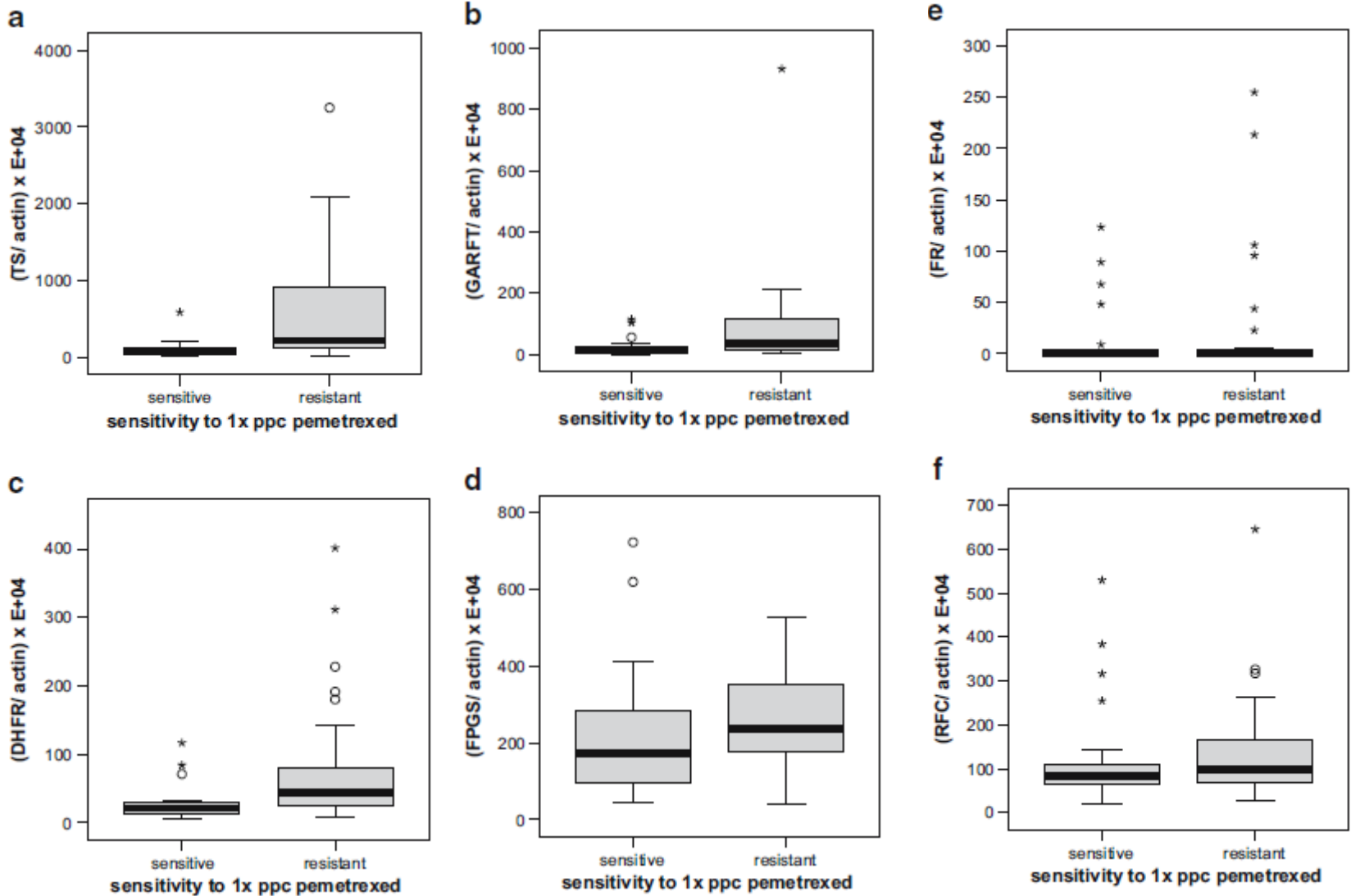
Inhibition of Folate Enzymes by Pemetrexed, Raltitrexed, and Methotrexate

Compound	TS (nM)	DHFR (nM)	GARFT (nM)
Pemetrexed †	109 ±9.0	7.0 ±1.9	9,300 ±690
Pemetrexed Glu5 †	1.3 ±0.3	7.2 ±0.4	65 ±16
Raltitrexed †	6.0 ±0.9	45 ±3	424,000
Raltitrexed Glu5 †	1.4 ±0.1	30 ±3	132,000
Methotrexate ‡	13,000	0.004	80,000
MTX Glu5 ‡	47	0.004	2,500

† Shih et al. *Cancer Res* 57:1116-1123, 1997

‡ Chabner et al. *J Clin Invest*, 76:907-912, 1985

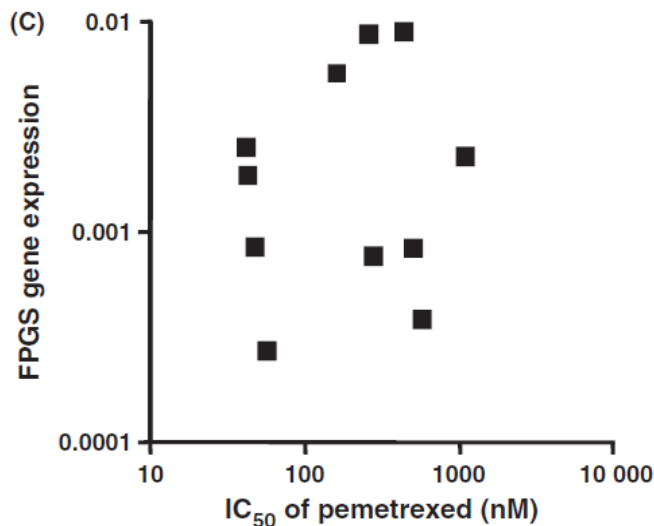
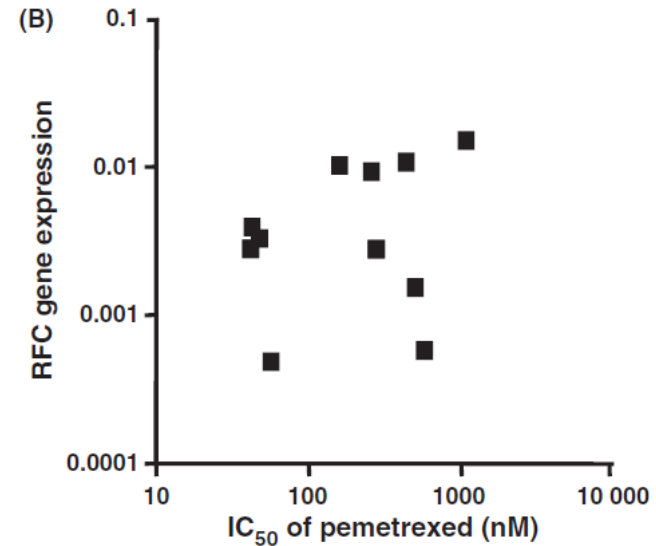
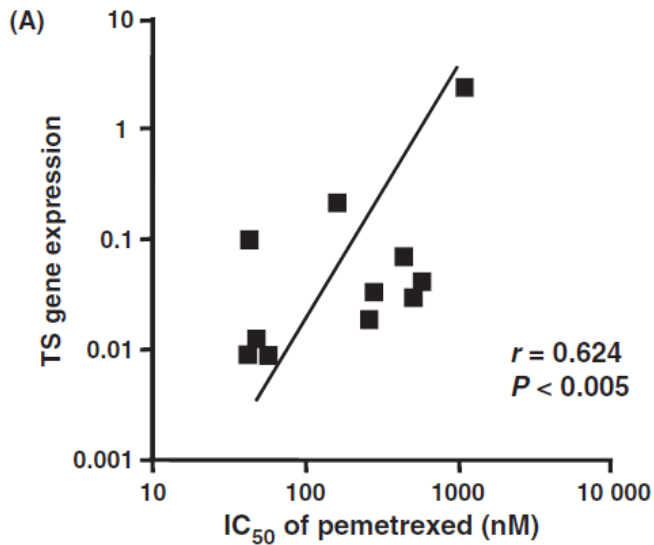
In vitro chemosensitivity of pemetrexed is correlated with target gene expression



Gene	mRNA expression*		
	Sensitive <i>n</i> =26	Resistant <i>n</i> =32	<i>P</i> -value
A			
TS	80	224	0.0003
GARFT	18	38	0.003
DHFR	21	45	0.001
FPGS	171	235	0.070
mrp4	3.5	6.2	0.026
mrp5	259	352	0.197
RFC	85	99	0.298
α-FR	0.005	0.039	0.247
B			
TS	55	176	0.002
GARFT	19	27	0.049
DHFR	17	37	0.008
FPGS	179	230	0.154
mrp4	3.5	5.0	0.096
mrp5	259	352	0.333
RFC	84	94	0.317
α-FR	0.006	0.038	0.455

mRNA expression in tumors grouped according to sensitivity or resistance to 100 µg/ml (A) or 10 µg/ml (B) of pemetrexed after 1 hour exposure

Relationship between Pemetrexed Sensitivity and TS, RFC and FPGS Expression in NSCLC Cell Lines



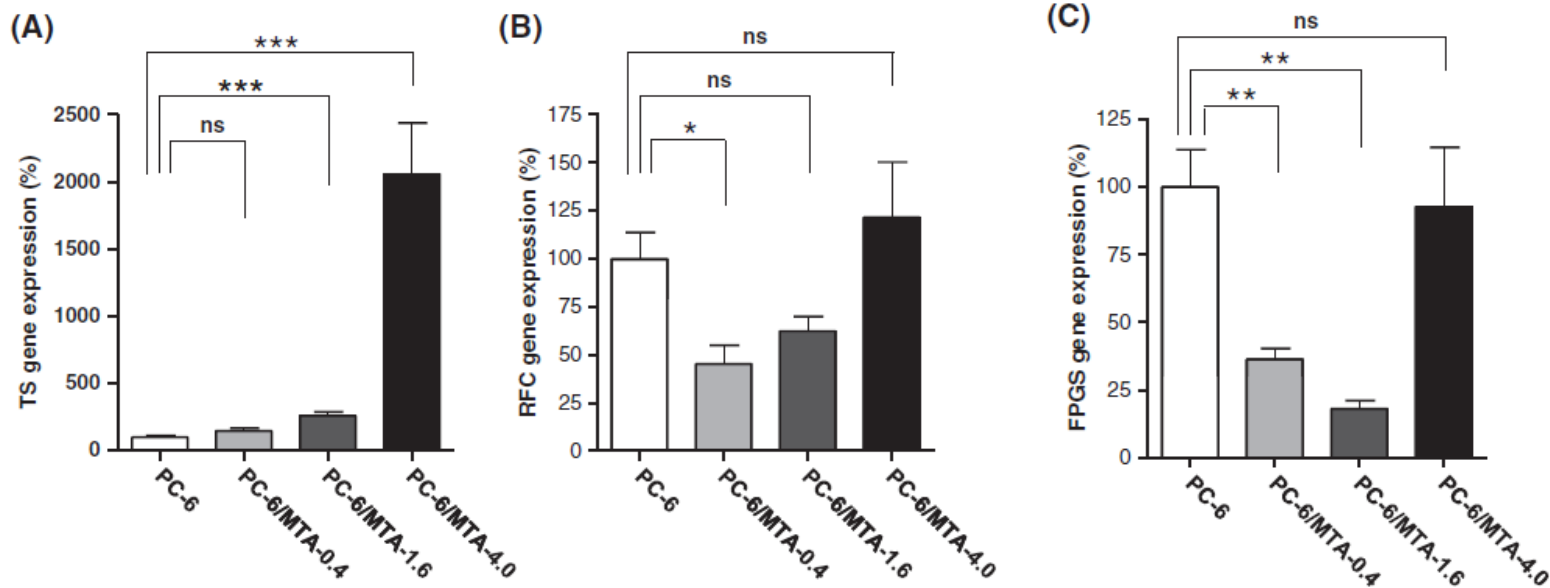
Cell Lines

- 8 ADC
- 2 SCC
- 1 LCC

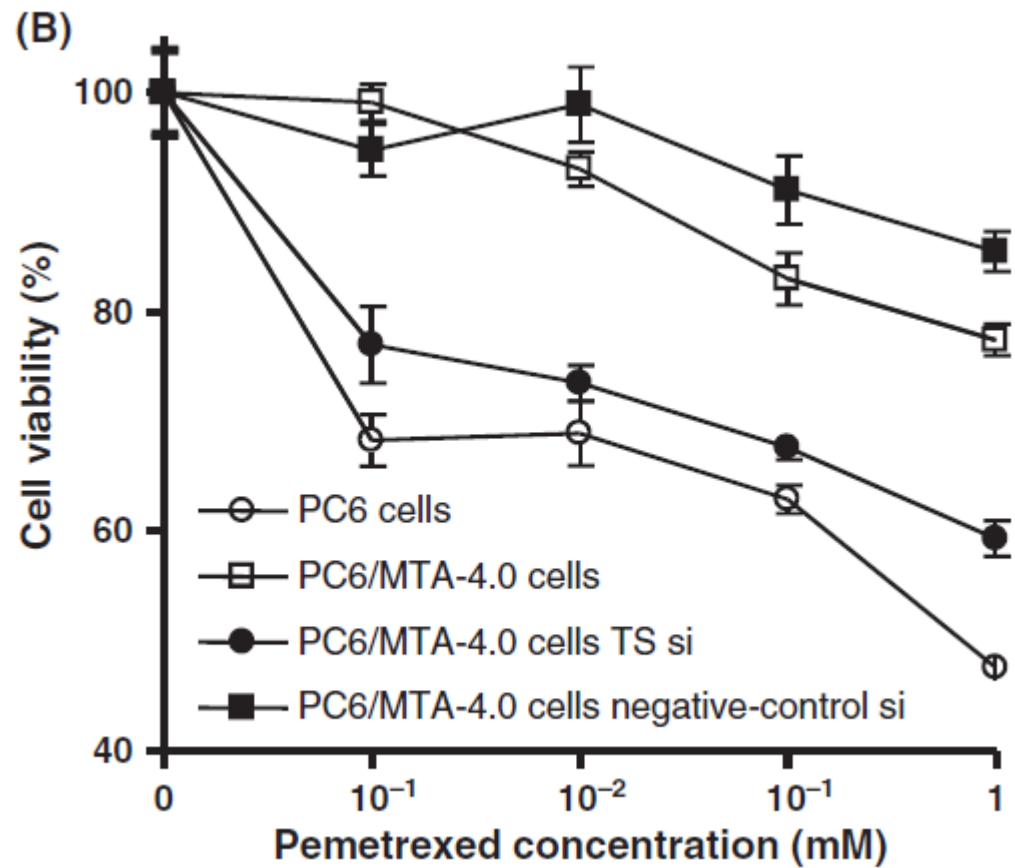
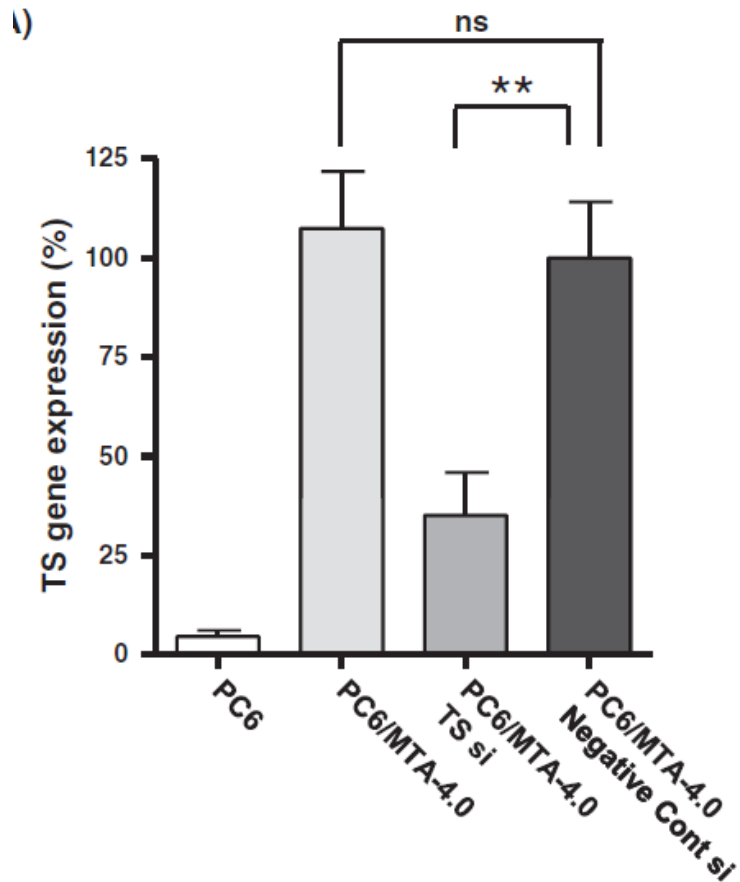
Significance of TS for Resistance to Pemetrexed in Lung Cancer

Cell lines	Pemetrexed IC ₅₀ (μM)	95% CI (μM)	RR
PC6	0.1	0.07–0.13	–
PC6/MTA-0.4	0.53	0.32–0.88	5.3
PC6/MTA-1.6	2.38	1.51–3.76	23.8
PC6/MTA-4.0	43.87	22.08–87.20	438.7

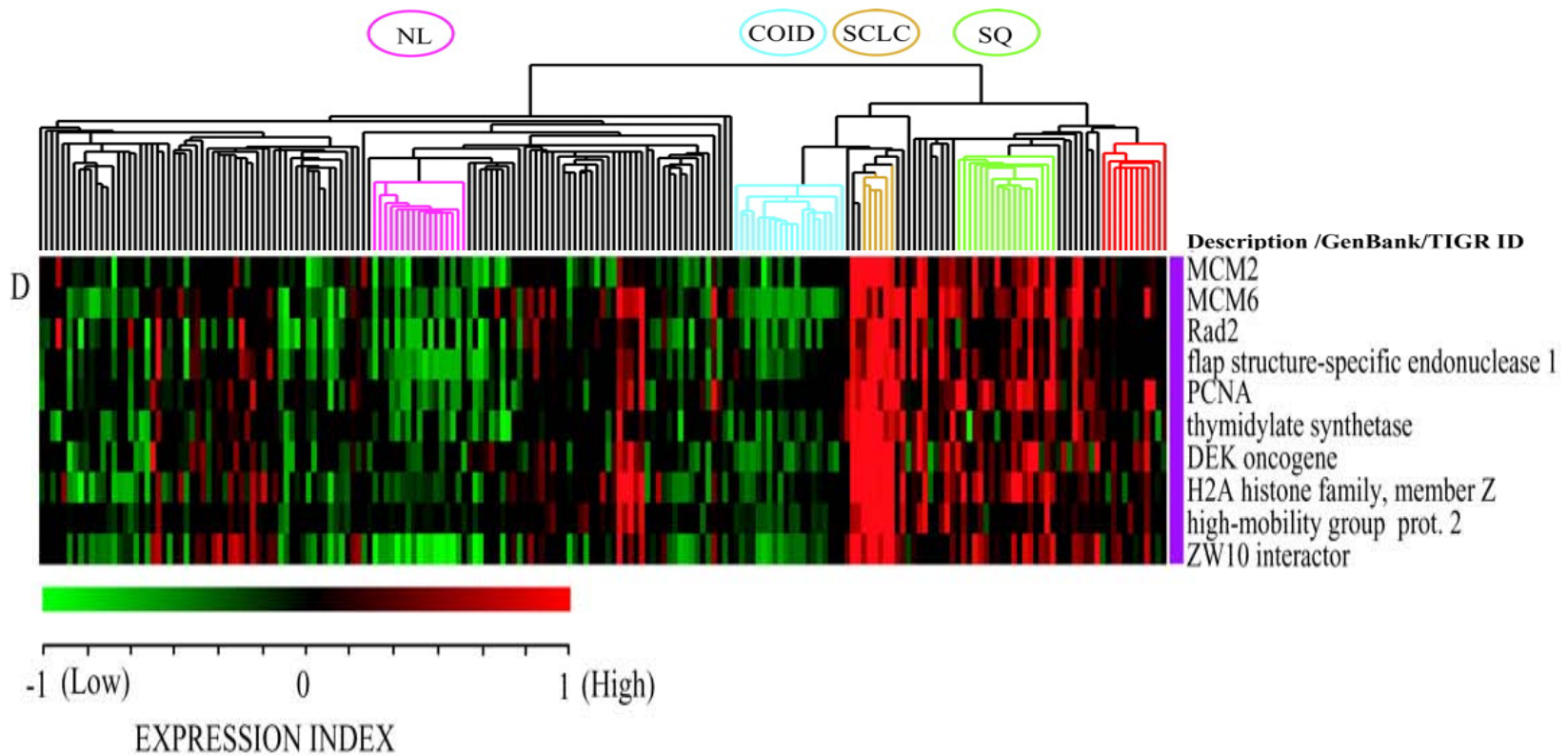
RR, resistance rate: (IC₅₀ in resistant subline)/(IC₅₀ in the parental subline).



Significance of TS for Resistance to Pemetrexed in Lung Cancer

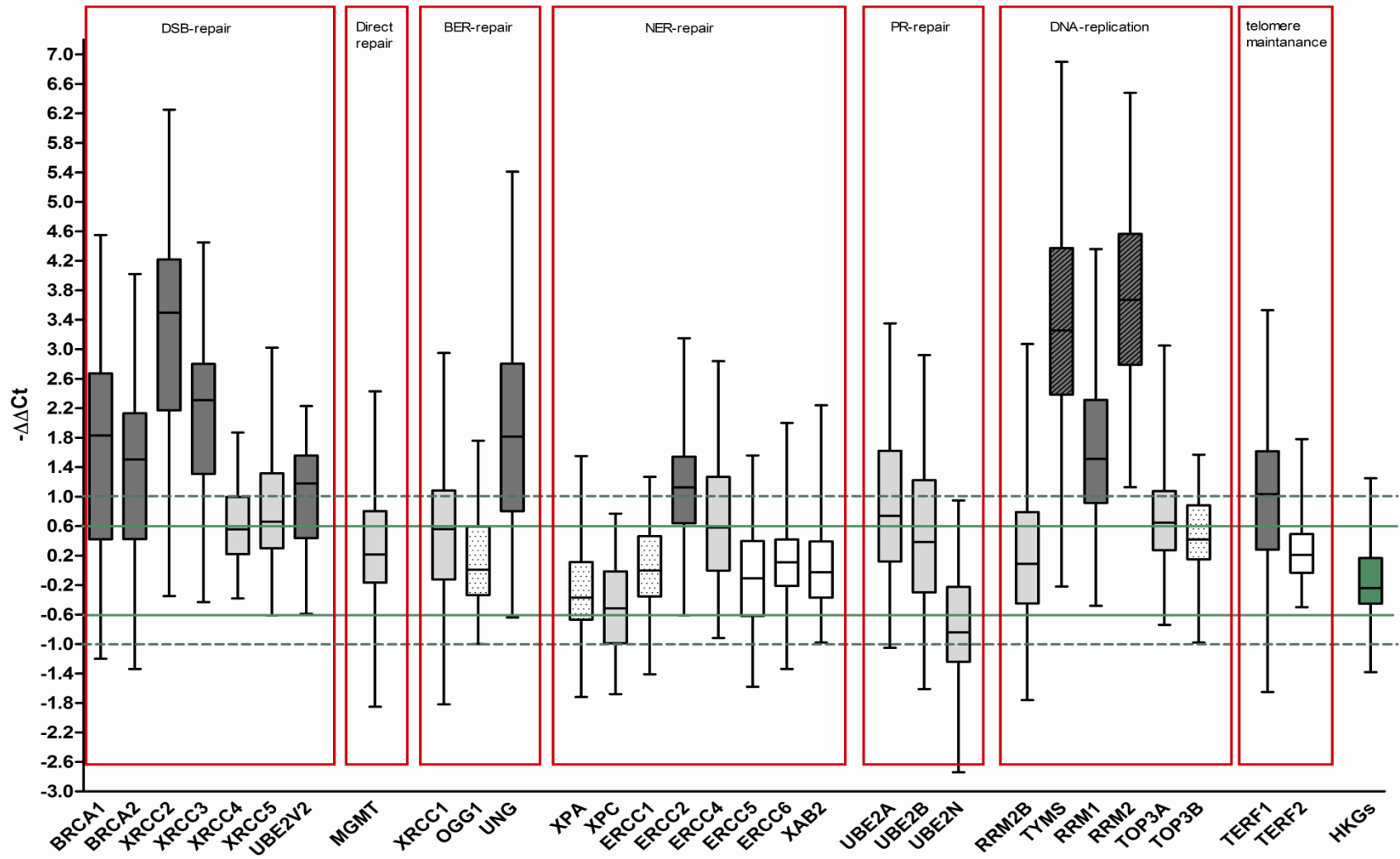


Classification of Human Lung Carcinomas by mRNA Expression Profiling Reveals Distinct Adenocarcinoma Subclasses



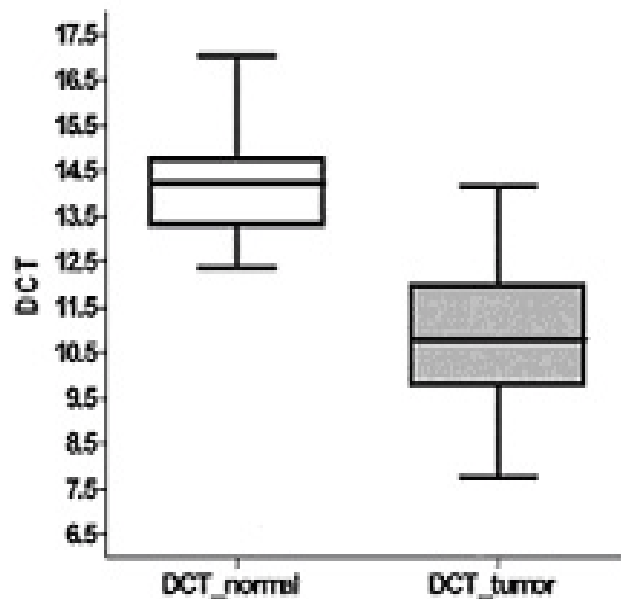
SCLC – Very High TS ; Squamous – Intermediate TS , Adenocarcinoma – Low TS

Gene Expression Modulation in 64 Cases of Resected NSCLC - DNA Repair Genes



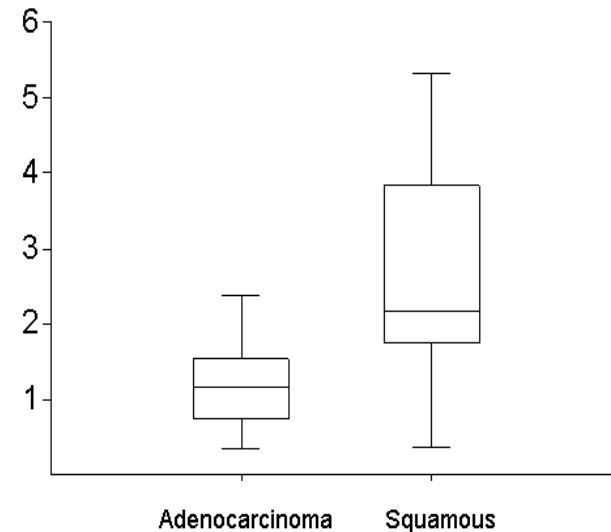
Thymidilate Synthase Expression in Normal Lung Tissue & Lung Cancer

Snap Frozen Tissues



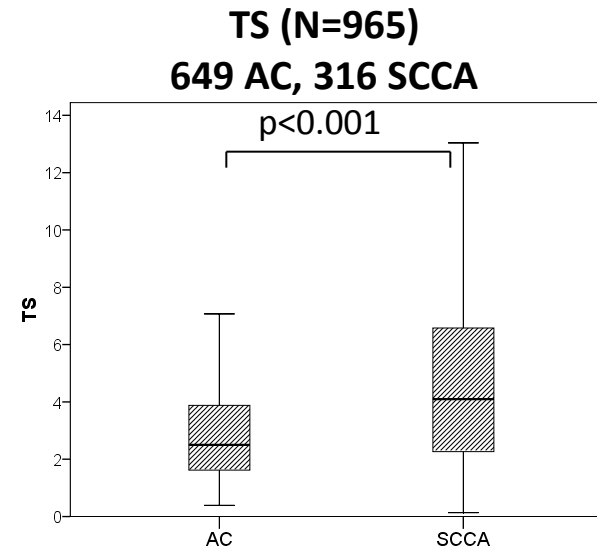
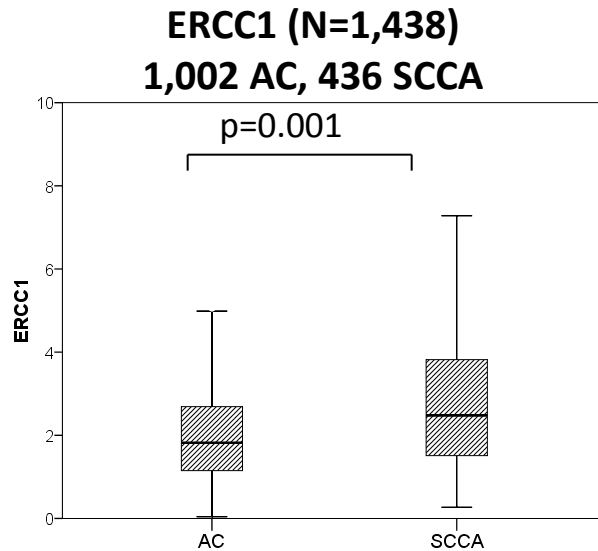
Significantly Higher in Lung Cancer than in normal lung tissue

FFPE Tissues



Significantly Higher in Squamous Cell Carcinoma of the Lung

Heterogeneity of mRNA Expression Levels in NSCLC: AC versus SCCA



ERCC1
(Reference <1.7
for platinum)

**% Below
Reference Level**

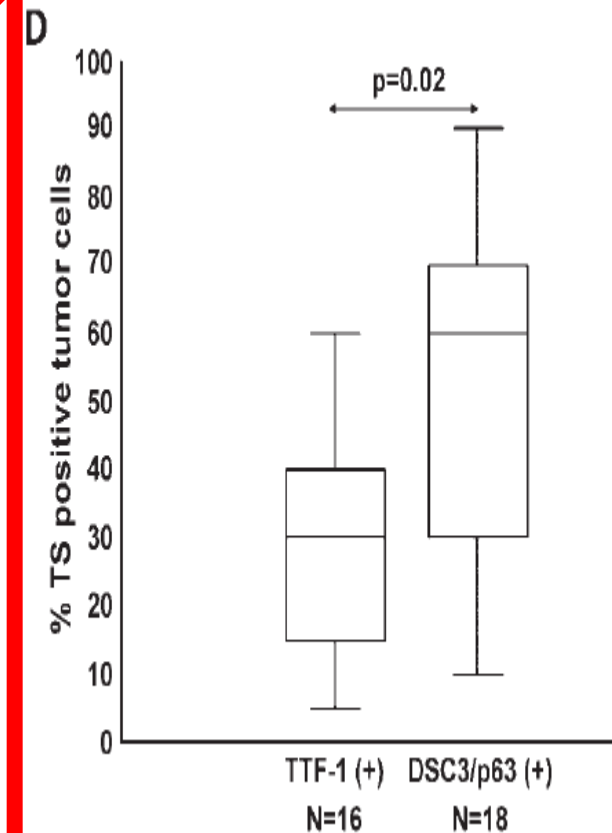
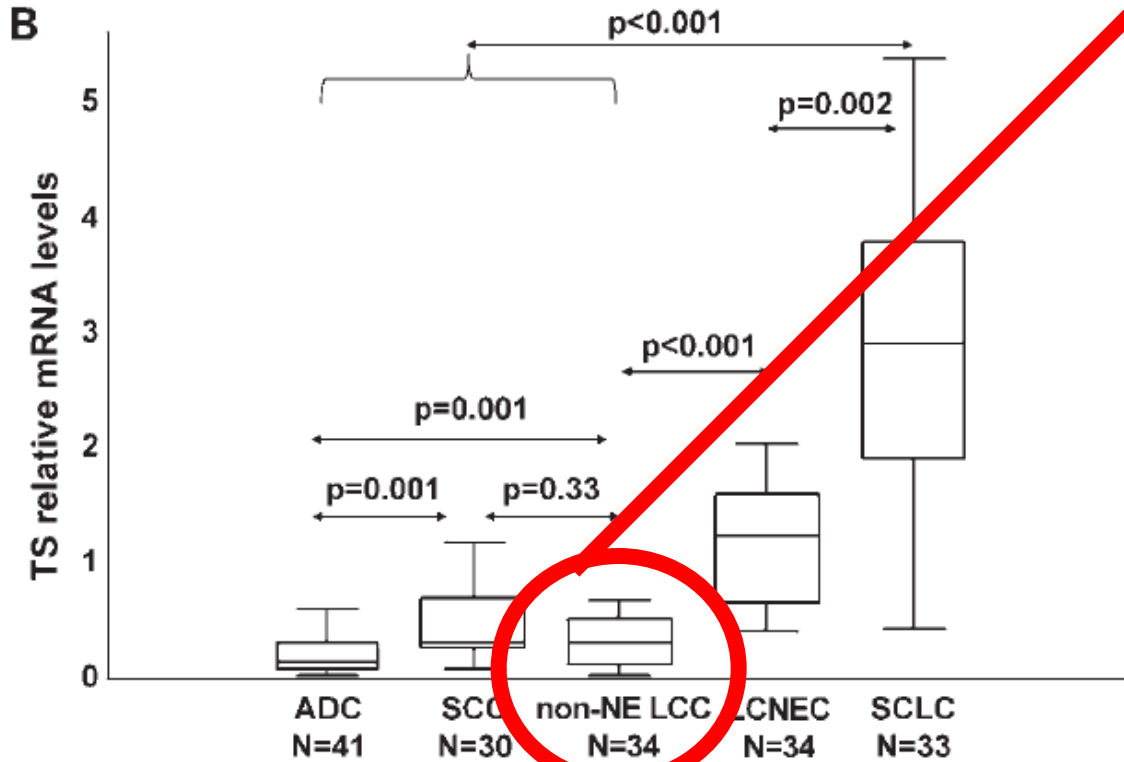
TS
(Reference <2.33
for pem)

**% Below
Reference Level**

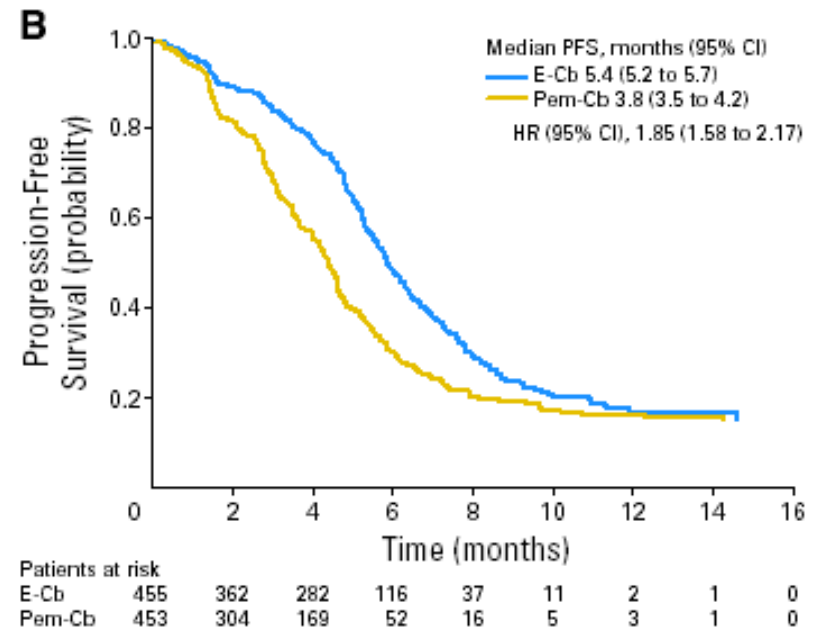
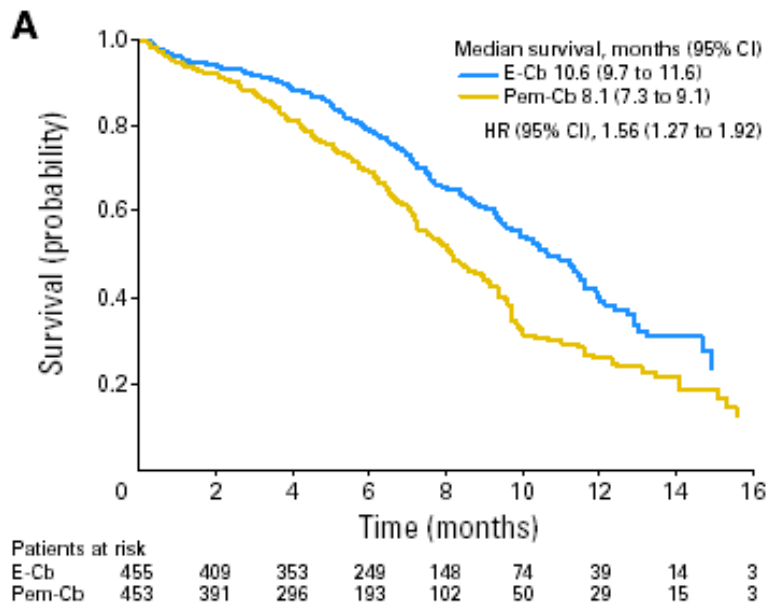
NSCLC-Total	43.4%
NSCLC-Adenoca	46%
NSCLC-SCCA	30.7%

NSCLC-Total	41.3%
NSCLC-Adenoca	45.7%
NSCLC-SCCA	25.9%

TS mRNA Expression in Lung Cancer (n=146)



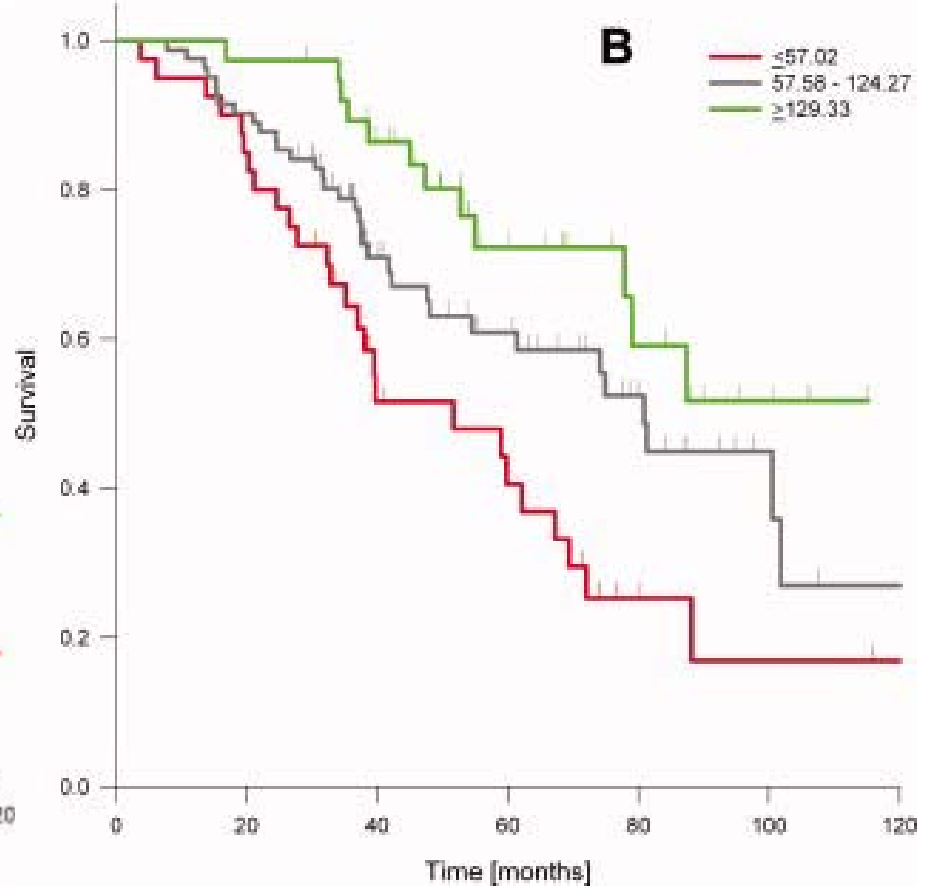
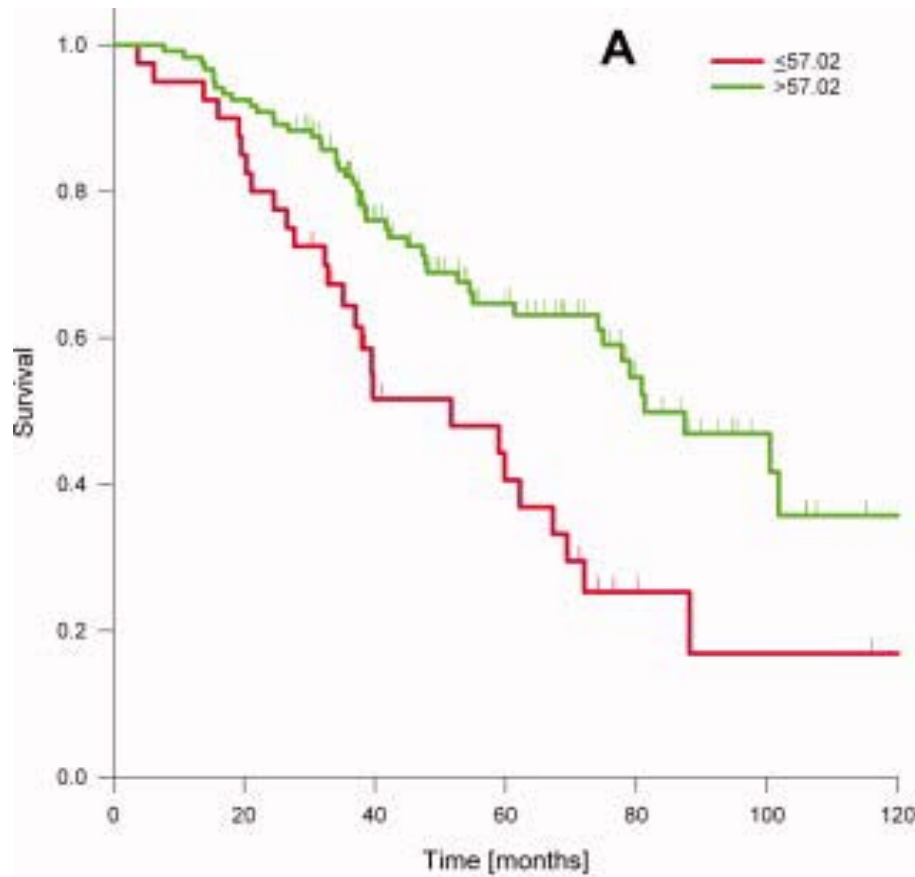
Phase III Study ED-SCLC of Pemetrexed/ Carboplatin vs Etoposide/ Carboplatin



TS Expression and Survival in Stage I NSCLC

- 160 patients with resected NSCLC (adeno+BAC 84, squamous 54, large cell 21)
- TS in situ protein quantification (AQUA)(n=160) & RT-PCR (N=85)
- The optimal cut-off point for TS protein at the 25% percentile
- TS mRNA not significantly associated with patient survival or TS protein expression
- Ts mRNA levels lower in adenocarcinoma compared to squamous cell carcinoma

TS Expression and Survival in Stage I NSCLC

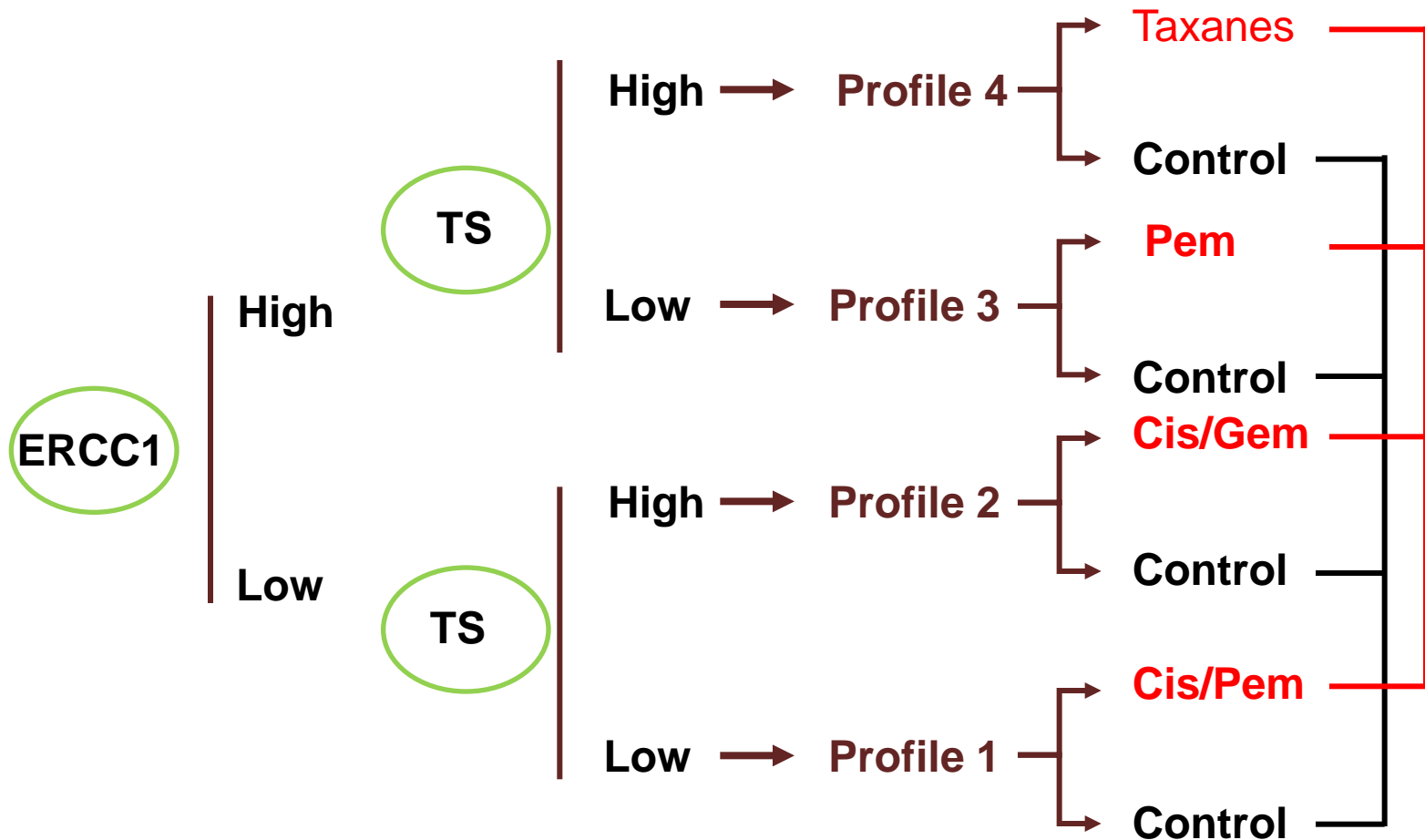


Predictive Molecular Markers of Neoadjuvant Gemcitabine and Pemetrexed in NSCLC

- 52 patients, stage I-III
- G 1500 mg/m² followed by P 500 mg/m² days 1,15,29,43
- Thoracotomy days 64-77
- RR 35% (95%CI:22-50), no pCR
- Pre- & post-treatment levels of RRM1 and TS available in 10 patients (p=0.025)
- Tumor response to CT was inversely correlated with the post-treatment levels of expression of RRM1 (p <0.001) and TS (p < 0.006). No significant difference in PFS and OS

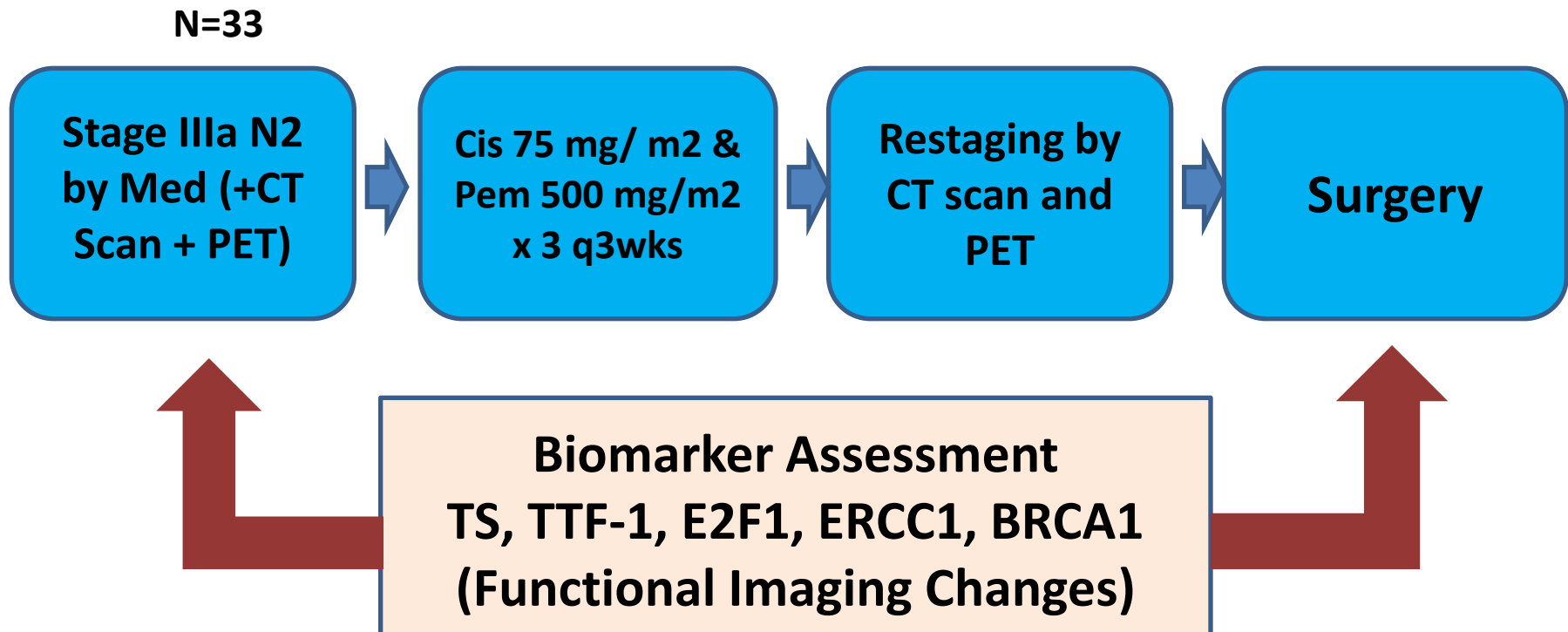
ITACA Adjuvant Trial

Pharmacogenomics: Yes or No?



Control = investigators' choice of cisplatin-based doublet
Primary endpoint = OS; Sample size = 700 patients

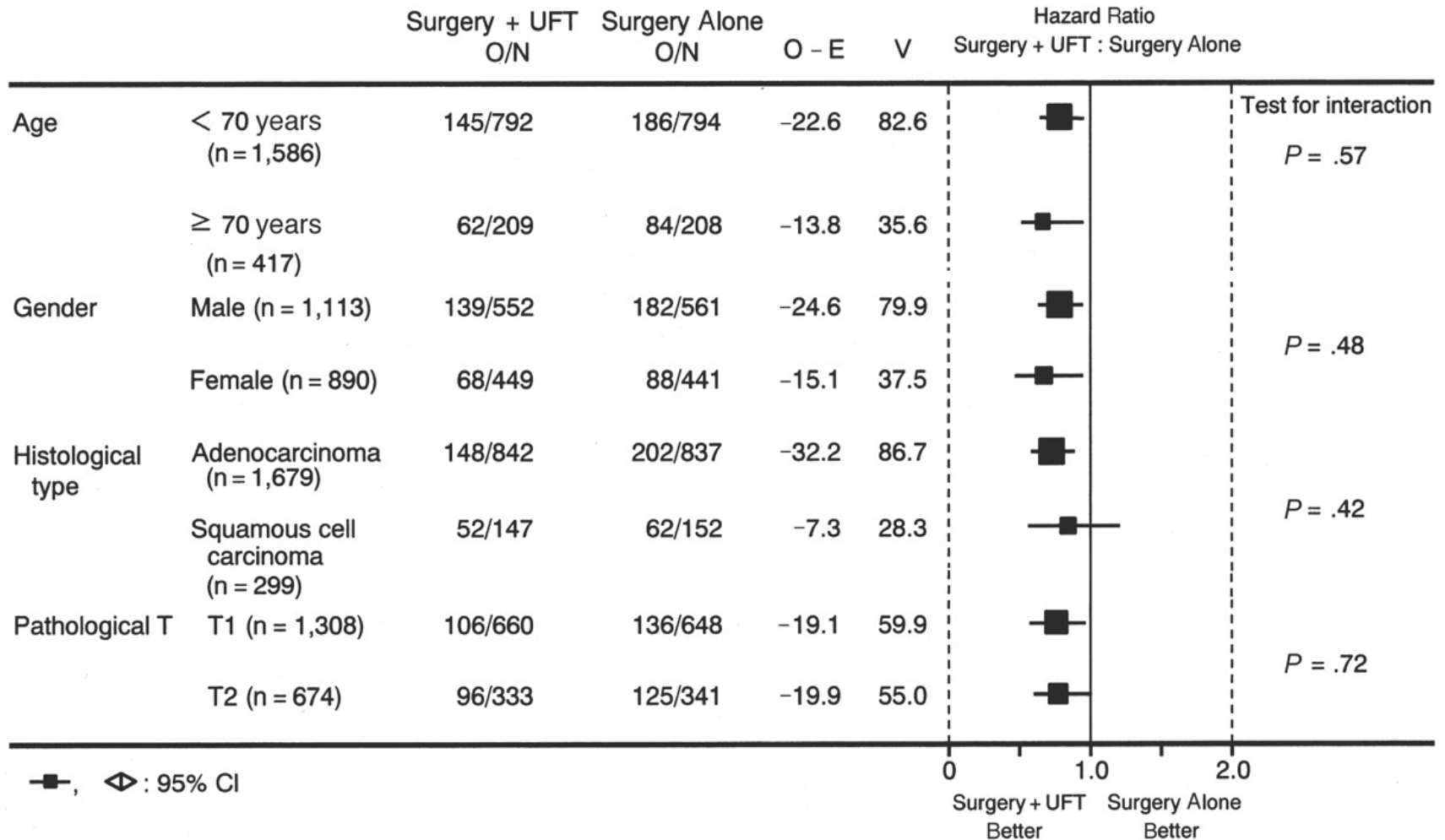
Phase II Study of Preoperative Cis/Pem in Stage IIIa N2 non-squamous NSCLC



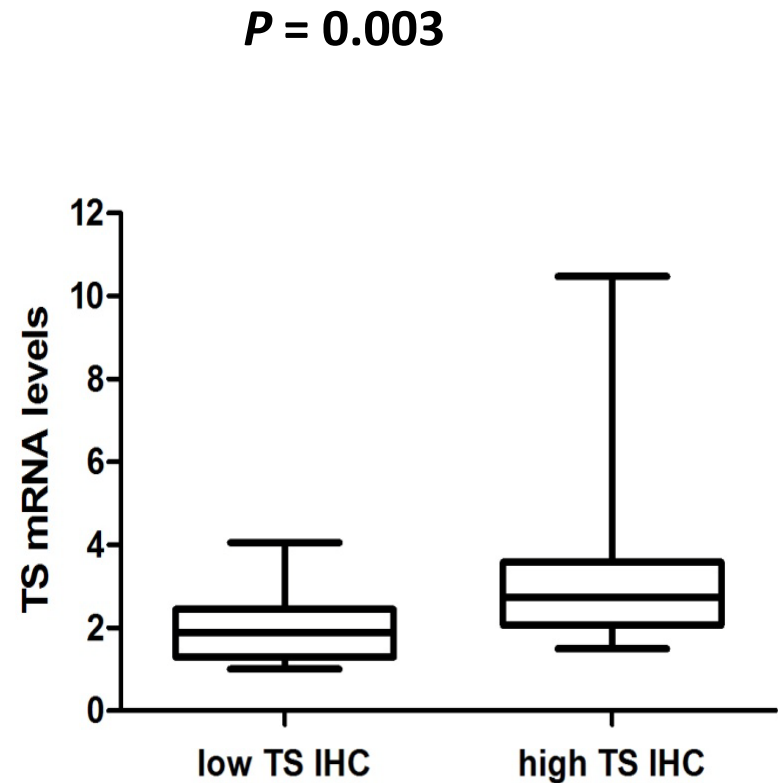
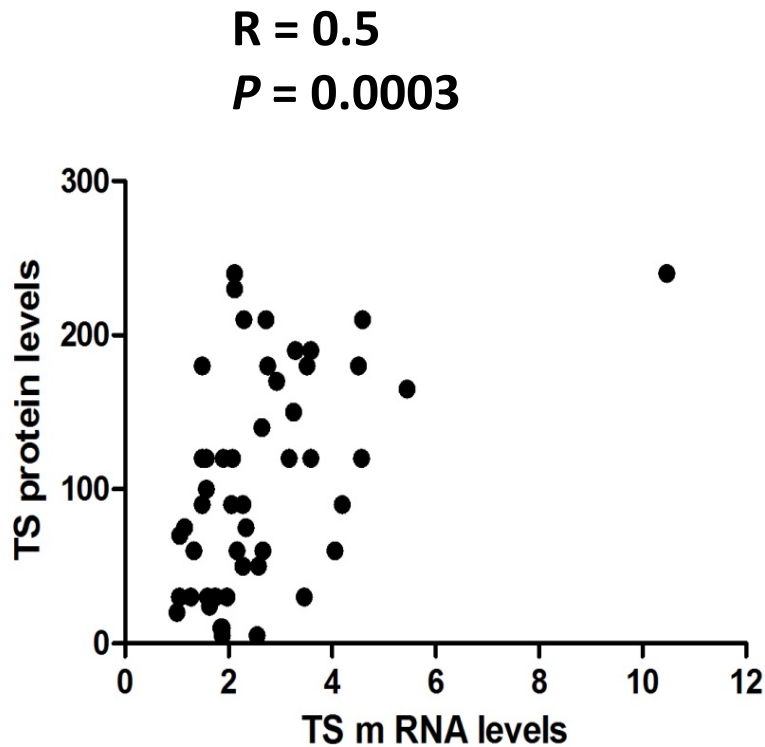
Primary end point : ORR

Secondary end points :pCR,PFS,OS, tumor downstaging, toxicity

Meta-Analysis of Postoperative Adjuvant CT with Tegafur-Uracil in NSCLC

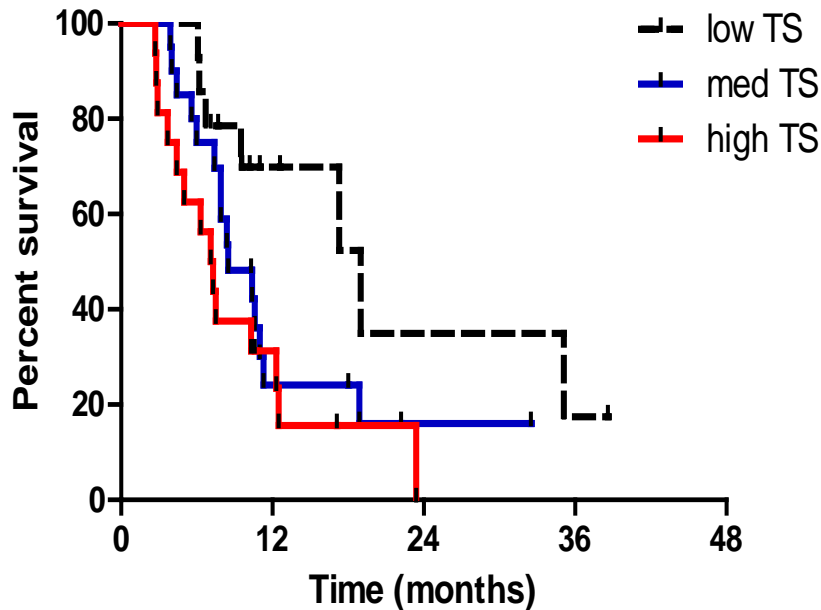


Correlation between Protein and mRNA TS Expression in 50 Advanced Stage MPM

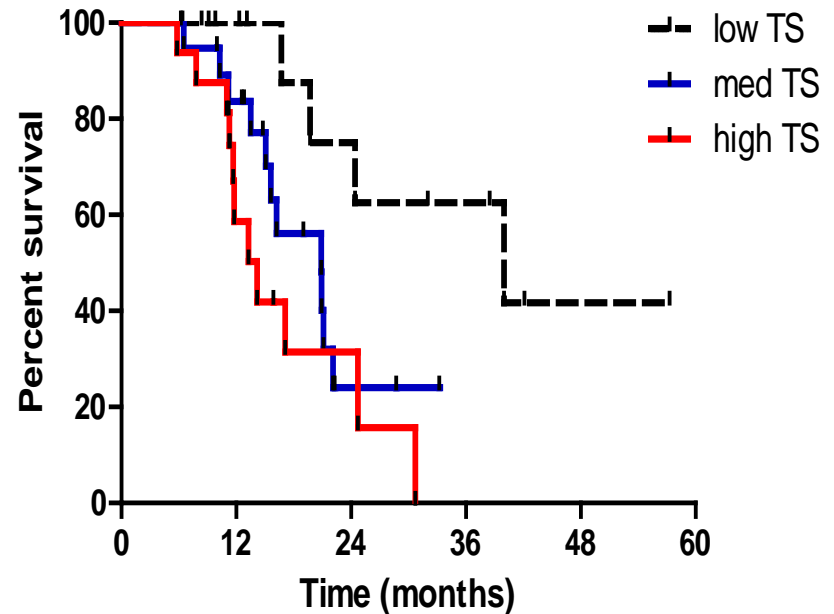


TS Tertile Levels and Clinical Outcome in 50 Advanced Stage MPM

TIME TO PROGRESSION



MEDIAN SURVIVAL



Low TS (n=14): 19 mo

Med TS (n=19): 8.5 mo ***p=0.02***

High TS (n=16): 7.2 mo

Low TS (n=14): 40 mo

Med TS (n=19): 20.9 mo ***p=0.007***

High TS (n=16): 14.2 mo

Conclusion

- **There is evidence of a in vitro correlation between TS levels and sensitivity to Pemetrexed.**
- **In specimens from chemo-naïve patients with NSCLC, TS expression was significantly higher in squamous cell carcinoma compared with adenocarcinoma.**
- **LCC immunoprofile may resemble that of squamous (DSC3 positive) or adeno- (TTF-1 positive) carcinomas. A strong correlation was observed between DSC3+/high TS and TTF-1+/low TS phenotypes, respectively.**
- **Good level of correlation of TS expression in cytological and corresponding histological samples**
- **These hypothesis-generating findings are worthy of prospective validation in ongoing studies**