

## Proteomics: 'prognostische factoren' mogelijk?

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## Gouden Standaard in mRCC

- Motzer/MSKCC Criteria
    - Verminderde Karnofsky score
    - Hoog LDH
    - Laag Hb
    - Hoog gecorrigeerd Calcium
    - Tijd diagnose tot start therapie (<12mnd)
- } Prognose:  
Slecht (4 mnd)  
Gemiddeld (10 mnd)  
Goed (20 mnd)



## Introductie

- Proteomics in gemetastaseerd niercarcinoom
- In Perspectief (*literatuur*)
- Vervolgonderzoek

## SELDI-TOF-Massa Spectrometrie

- **Kritiek**
  - reproduceerbaarheid
  - Verschillen in gevonden biomarkers
  - Verschil in procedure
  - Analytische methode

Validatie is noodzakelijk!

=> niet direct klinisch toepasbaar

Wel als screeningsinstrument



## Methodologie

- Screenings methode: SELDI-TOF MassSpec
- Validatie methode: ELISA, Immunoturbidimetry
- ‘Gouden Standaard’ MSKCC prognostisch model
- Nieuwe survival modellen
- Conclusies



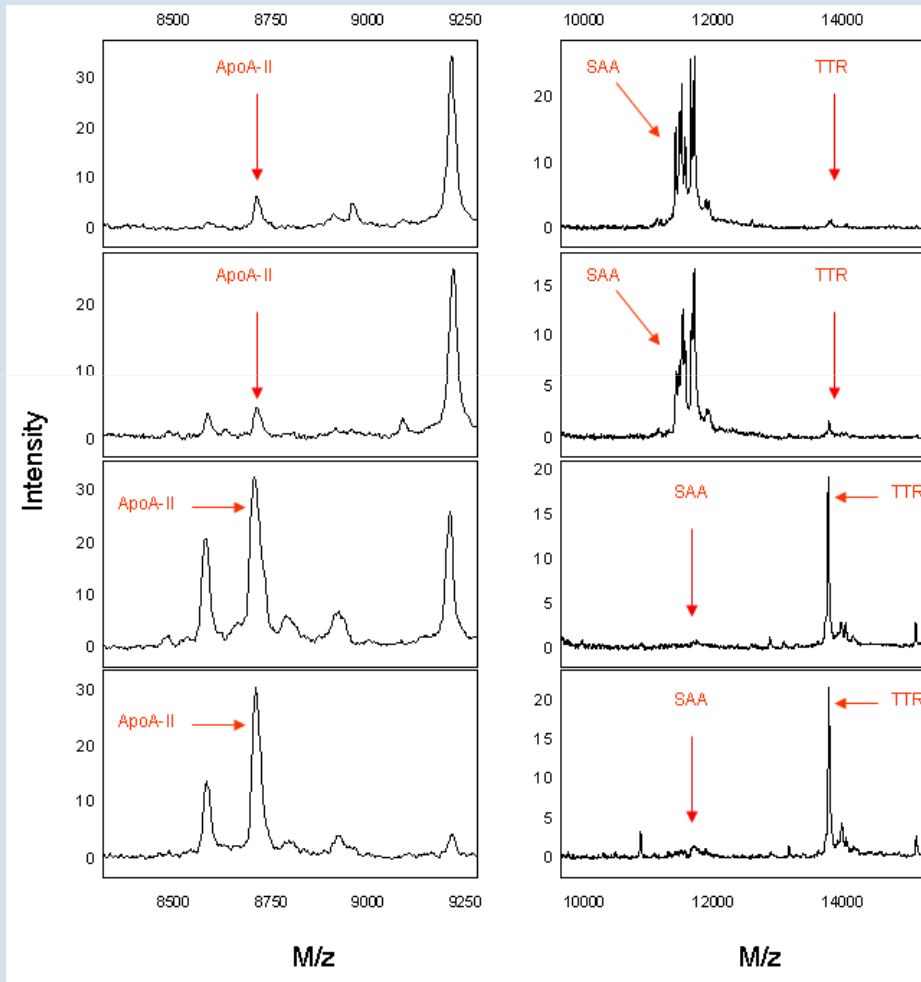
## Patients & Methods

- 114 gemetastaseerde Clear Cell RCC patients , Lft ~60jaar
  - UMC Utrecht: N=63
  - AvL A'dam: N=29
  - EMC R'dam: N=22
- 1<sup>st</sup> lijn: Interferon-gebaseerde therapie (73%)
- 2e lijn: TKI (17%)
- Alle prognosticators voorspellend: Hb, LDH, Calcium etc.
- Serum samples



2nd* experiment m/z ratio (kDa)	Identified protein	References	P †
4.11	not identified yet		0.02
4.49	not identified yet ‡		0.02
4.61	not identified yet		0.7
4.84	not identified yet		0.9
5.66	not identified yet		0.6
5.93	fibrinogen alphaE fragment	Nomura et al., <i>Proteomics</i> , 4, 2004	0.9
6.46	Apolipoprotein C-1 fragment	?	0.02
6.66	Apolipoprotein C-I	Engwegen et al., <i>Trends in Pharm. Sciences</i> , Vol 27, No.5, 2006	0.2
6.68	not identified yet §		<0.001
7.80	Platelet Factor-4	Trocme et al., <i>British Medical Journal</i> , Aug 2008	0.6
8.00	not identified yet		0.2
8.24	Platelet-associated PF-4	Cervi et al., <i>Blood</i> , Volume 111, No.3, 2008	0.5
8.61	Apolipoprotein A-II	Ehmann et al. <i>Pancreas</i> , Volume 34, No.2, 2007	<0.001
<b>8.72</b>	<b>Apolipoprotein A-II</b>	Ehmann et al. <i>Pancreas</i> , Volume 34, No.2, 2007	<b>0.003</b>
8.95	Apolipoprotein A-II	Hartmann et al., <i>Pancreas</i> , Volume 35, No.4, 2007	0.3
9.21	Haptoglobin 1-alpha	Tolson et al. <i>Laboratory Investigation</i> 84, 2004	0.5
9.32	not identified yet		0.1
9.37	not identified yet		0.9
9.44	Apolipoprotein C-III	Chen et al. <i>Journal of Chromatography A</i> , 1162, 2007	0.7
<b>11.71</b>	<b>serum amyloid alpha</b>	Engwegen et al., <i>Trends in Pharm. Sciences</i> , Vol 27, No.5, 2006	<b>&lt; 0.001</b>
<b>13.79</b>	<b>Transthyretin</b>	Engwegen et al., <i>Trends in Pharm. Sciences</i> , Vol 27, No.5, 2006	<b>&lt; 0.001</b>
15.10	alpha Globin	Tolson et al. <i>Laboratory Investigation</i> 84, 2004	0.3
15.98	Hemoglobin beta	Tolson et al. <i>Laboratory Investigation</i> 84, 2004	0.2
16.19	Hemoglobin beta + SPA adduct	Tolson et al. <i>Laboratory Investigation</i> 84, 2004	0.8
18.41	not identified yet		0.2
23.49	not identified yet		0.3
25.01	not identified yet		0.001
28.13	Apoprotein A-I	Engwegen et al., <i>Trends in Pharm. Sciences</i> , Vol 27, No.5, 2006	0.01
33.33	Osteopontin	Hartmann et al., <i>Pancreas</i> , Volume 35, No.4, 2007	0.2
48.12	not identified yet		0.3
67.17	Albumin	Sundsten et al, <i>Proteome Science</i> , 4:22, 2006	0.1

## Eiwit Profiel mRCC patients



Slechte overlevers < 6 mnd

Slechte overlevers < 6 mnd

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Goede overlevers > 36 mnd

Goede overlevers > 36 mnd

## Apolipoprotein A2

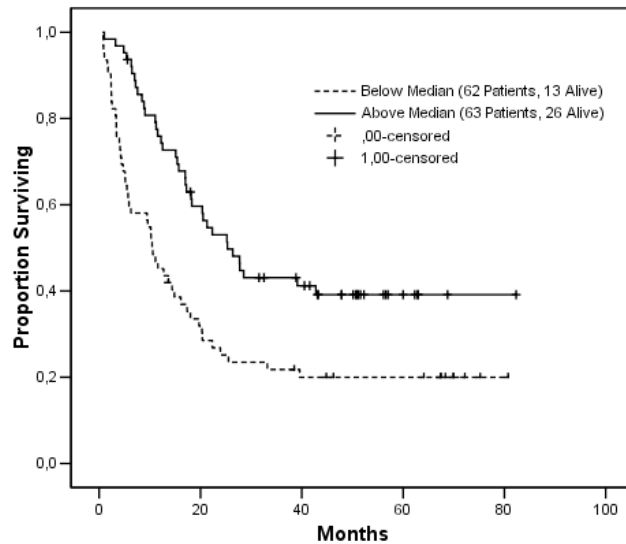
### Screening

Continue variabele:  
 $p = 0.00051$

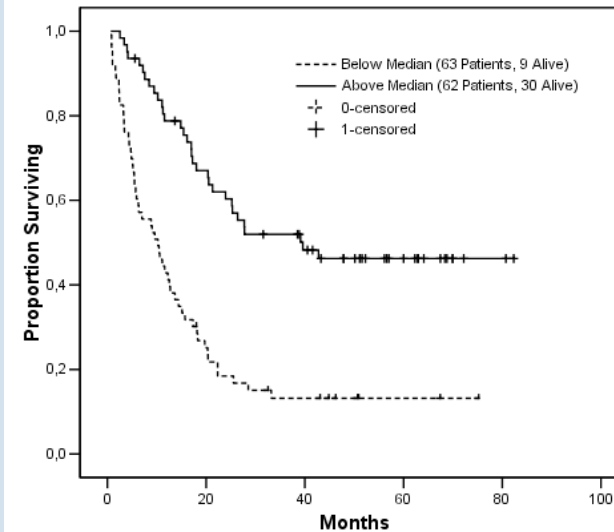
### Validation

Continue variabele:  
 $p = 5.5 \times 10^{-9}$

SELDI-TOF Mass Spectrometry  
 Mass Peak: 8.5 kD



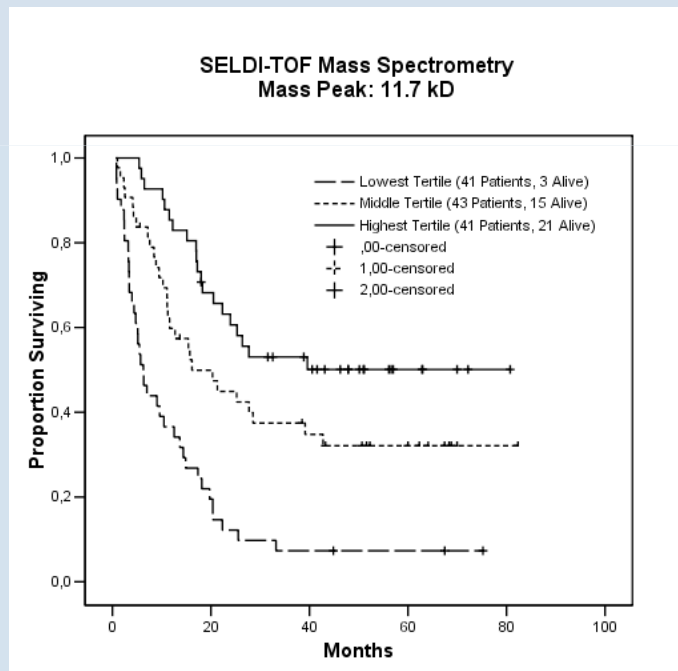
Immunoturbidimetry  
 Apolipoprotein A-2



## Serum Amyloid Alpha

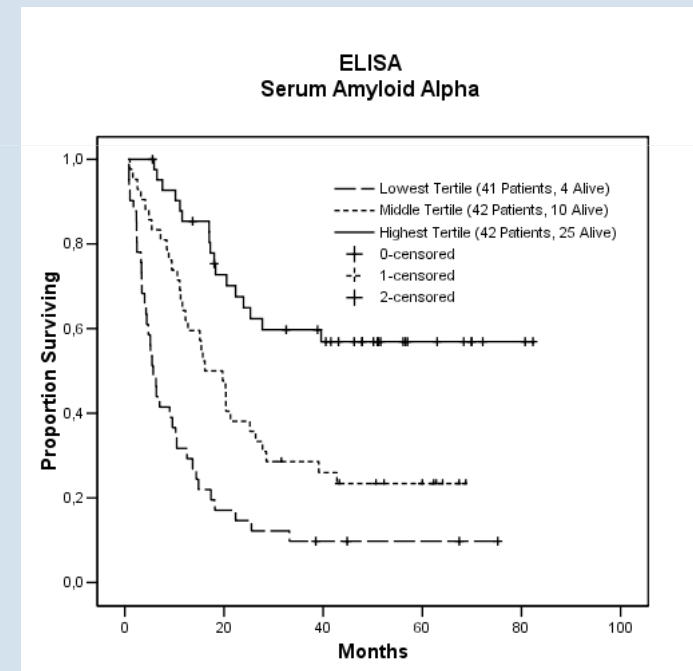
### Screening

Continue variabele:  
 $p = 2.0 \times 10^{-6}$



### Validation

Continue variabele:  
 $p = 1.1 \times 10^{-7}$





## Berekenen Survival Models

- Stapsgewijze benadering: Cox Proportional Hazard Regressie
- LLR = Log likelihood ratio:  
definieert de best geschatte waarschijnlijkheidsscore
- AIC = Akaika's Information Criteria  
*"de maat hoe goed de data past in een statistisch model"*

**====> AIC and LLR maatgevend**



## Multivariate Analysis

Alleen eiwitten => model van ApoA2 en SAA  
AIC = 732, P= 5.2x10<sup>-10</sup>

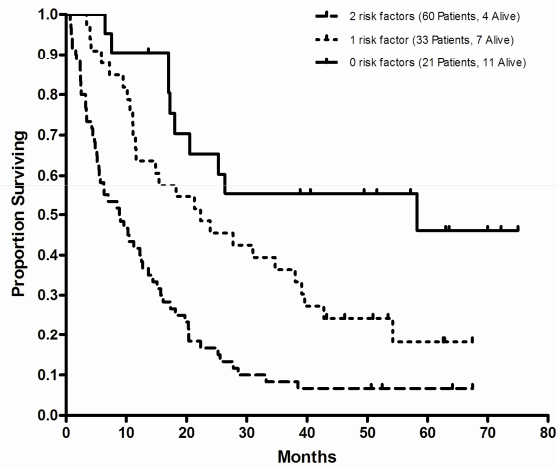
**Table 4 - Results of Multivariable Analysis**

<i>Factor</i>	<i>Poor Prognostic Category</i>	<i>Parameter Estimate</i>	<i>SE</i>	<i>P</i>	<i>Hazard Ratio</i>	<i>95% CI</i>
Karnofsky Performance Status	≤ 80%	0.83	0.26	0.00047	2.3	1.4 - 3.7
Lactate Dehydrogenase	> 1.5x upper limit of reference range	1.28	0.26	0.00073	3.6	1.7 - 7.5
Number of Metastatic Sites	2 or 3	0.56	0.38	0.032	1.8	1.1 - 2.9
Apolipoprotein A-II	≤ highest tertile (≤309 mg/L)	0.65	0.24	0.014	1.9	1.1 - 3.2
Serum Amyloid Alpha	> lowest tertile (>19.2 ng/mL)	0.71	0.26	0.0063	2.0	1.2 - 3.4
<i>All variables included</i>		AIC = 712		P = 1.4 x 10 <sup>-10</sup>		

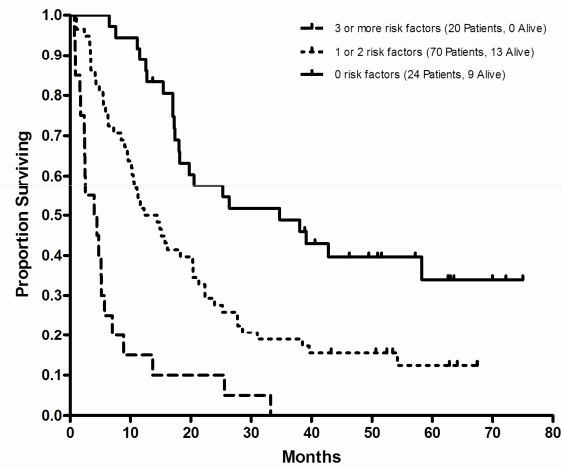


## Kaplan-Meier Curves

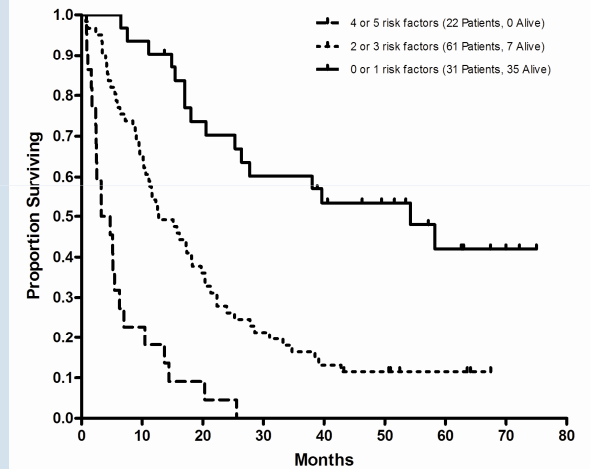
(A) Two-Protein Signature based on ApoA2 and SAA  
( $AIC=732, p=5.2 \times 10^{-7}$ )



(B) MSKCC Model corresponding to Motzer *et al.* 2002  
( $AIC=729, p=1.3 \times 10^{-7}$ )



(C) Novel Protein-Based Risk Model  
( $AIC=713, p=4.3 \times 10^{-11}$ )





**Table 5a - Novel protein-based models compared to prior defined categorized survival models**

Survival Model	Risk Factors	AIC	P	Risk Factors	No. Patients	Median OS (months)	Hazard Ratio	95% CI
Motzer <i>et al.</i> , JCO 2002 (MSKCC Risk Model)	Hb, LDH, corrected Calcium, KPS and Time from Diagnosis to Study Entry	729	$1.3 \times 10^{-7}$	0 †	24	38.0	—	—
				1 or 2	70	15.7	2.0	1.2 - 3.6
				≥3	20	4.0	7.7	3.9 - 25.4
Two-Protein Signature	ApoA2 and SAA	732	$5.2 \times 10^{-7}$	0 †	21	58.3	—	—
				1	33	22.3	2.1	1.0 - 4.3
				2	60	8.8	4.7	2.4 - 9.3
MSKCC Model expanded with ApoA2 and SAA	Hb, LDH, corrected Calcium, KPS, Time from Diagnosis to Study Entry, ApoA2 and SAA	724	$1.3 \times 10^{-8}$	0 or 1 †	27	42.8	—	—
				2 or 3	46	17.4	2.5	1.4 - 4.6
				≥4	41	5.2	5.9	3.1 - 11.0
Novel Protein-Based Model	LDH, KPS, Number of Metastatic Sites, SAA and ApoA2	713	$4.3 \times 10^{-11}$	0 or 1 †	31	54.2	—	—
				2 or 3	61	12.7	3.2	1.8 - 5.6
				≥4	22	3.3	11.2	5.6 - 22.4



**Table 5c - Comparison of MSKCC risk model and Novel Protein-Based Model**

<b>MSKCC Risk Groups</b>	<b>Total</b>			<b>Novel Protein-Based Risk Groups</b>								
	Patients (No.)	(% Survived	Median Survival (months)	Favorable			Intermediate			Poor		
				Patients (No.)	(% Survived	Median Survival (months)	Patients (No.)	(% Survived	Median Survival (months)	Patients (No.)	(% Survived	Median Survival (months)
Favorable	24	38	38.0	13	62	58.3	11	9	18.2	0	—	—
Intermediate	70	19	15.7	18	39	27.7	44	14	12.2	8	0	5.4
Poor	20	0	4.0	0	—	—	6	0	4.4	14	0	2.4
<i>Total</i>	<i>114</i>			<i>31</i>	<i>48</i>	<i>54.4</i>	<i>61</i>	<i>12</i>	<i>12.7</i>	<i>22</i>	<i>0</i>	<i>3.3</i>

43 patients (38%) switched between risk groups



## In Perspective

- **Serum Amyloid Alpha (SAA): Review, dec 2009, Malle et al.**
  - > GAP... 80's .... 90's .....2006 ??
  - verhoogd bij inflammatie en chronische ziekte (amyloidosis en cancer)
  - geproduceerd door lever en tumorcellen
  - verhoogd aanwezig in meerdere kankersoorten
  - verhoogd voorspelt slechtere ziektestadiering / overleving
  
- **JCO May 2009, Findeisen et al.**
  - Screening: MALDI-TOF MS
  - SAA voorspellend voor OS in melanoma patients
  - SAA discrimineert tussen Stage I and III/IV
  - NB: CRP en SAA onafhankelijke prognosticators
  
- **JCO May 2009, Pierce et al.**
  - Verhoogd SAA en CRP (onafhankelijk) geassocieerd met slechte OS & DFS in borstkanker patients



## Eindconclusies

- SELDI-TOF MS succesvol als robuuste screeningsmethode
- Goede validatie met conventionele techniek
- Combineren APOA2 en SAA ==> sterk survival model
- APOA2 en SAA verbeteren MSKCC model  
==> novel designed model
- Therapeutische keuze zou mogelijk verschillend zijn in 38% van pt'en



## Vervolgonderzoek

### Doel

- Validatie prognostische waarde ApoA2 en SAA in mRCC pts (TKI's)
- ApoA2 en SAA verbetert MSKCC?
- Vroege verandering in eiwitpiegels in tijd voorspelt response

### Methodologie

- Trainingsset, N=60 mRCC TKI-behandelde mRCC patients
  - Baseline, 4 weken en 8 weken (sommigen 16 weken)
  - Bepalen reële afkapwaarde etc.
- Validatieset, N=60 patients



## RELEVANTIE

1. Goede implementatie in kliniek mogelijk
2. Optimalizatie voor patienten, mn verbeterde therapiekeuze

NB: subklinische inflammatie

## TOEKOMSTIG PERSPECTIEF

1. Associeer eiwitten met OS in andere kankersoorten  
(*e.g. ovarian-, colorectal- and prostaat kanker*)
2. Voorspellende waarde van deze eiwitten voor therapieresponse?  
(*e.g. TKI's in mRCC patients*)



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- AMC: *Tatjana Niers, Dick Richel*
- VUMC: *Astrid van der Veldt, Fons vd Eertwegh, Epie Boven, Henk Verheul*
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