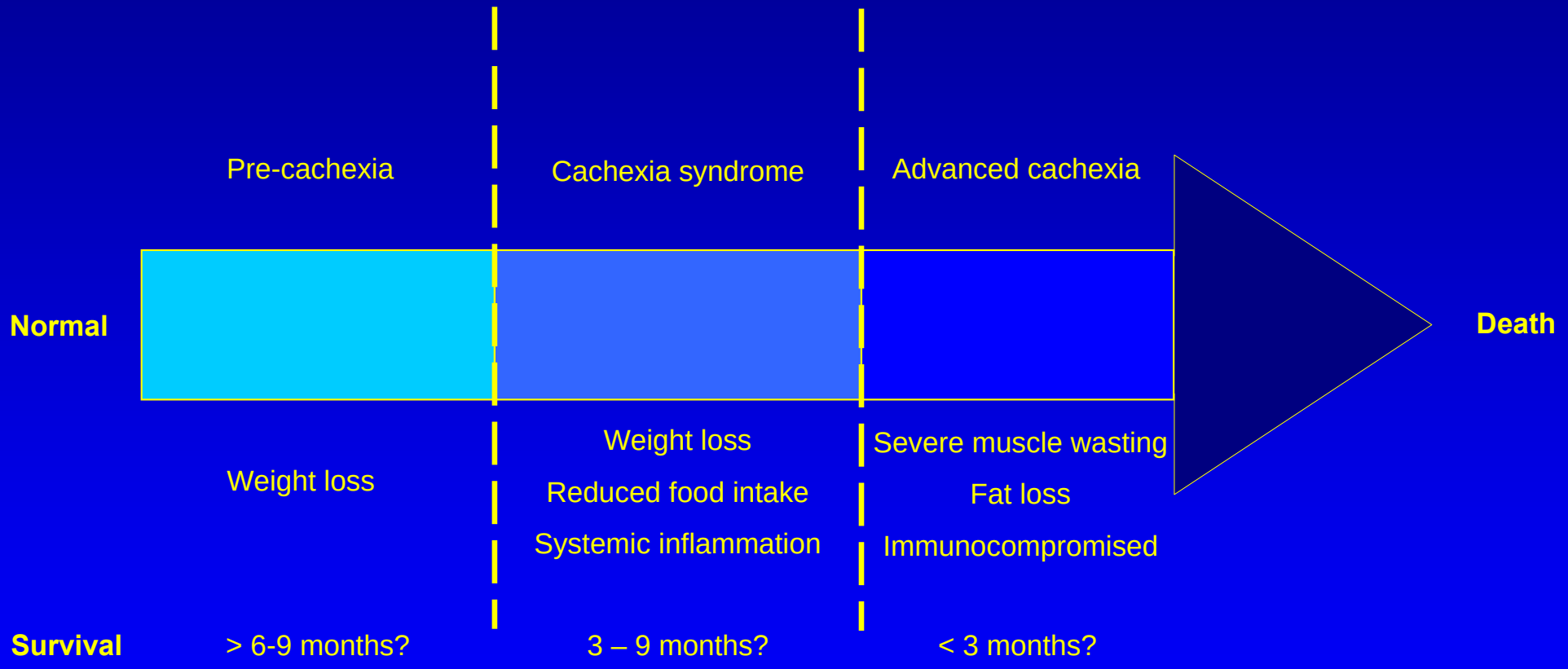
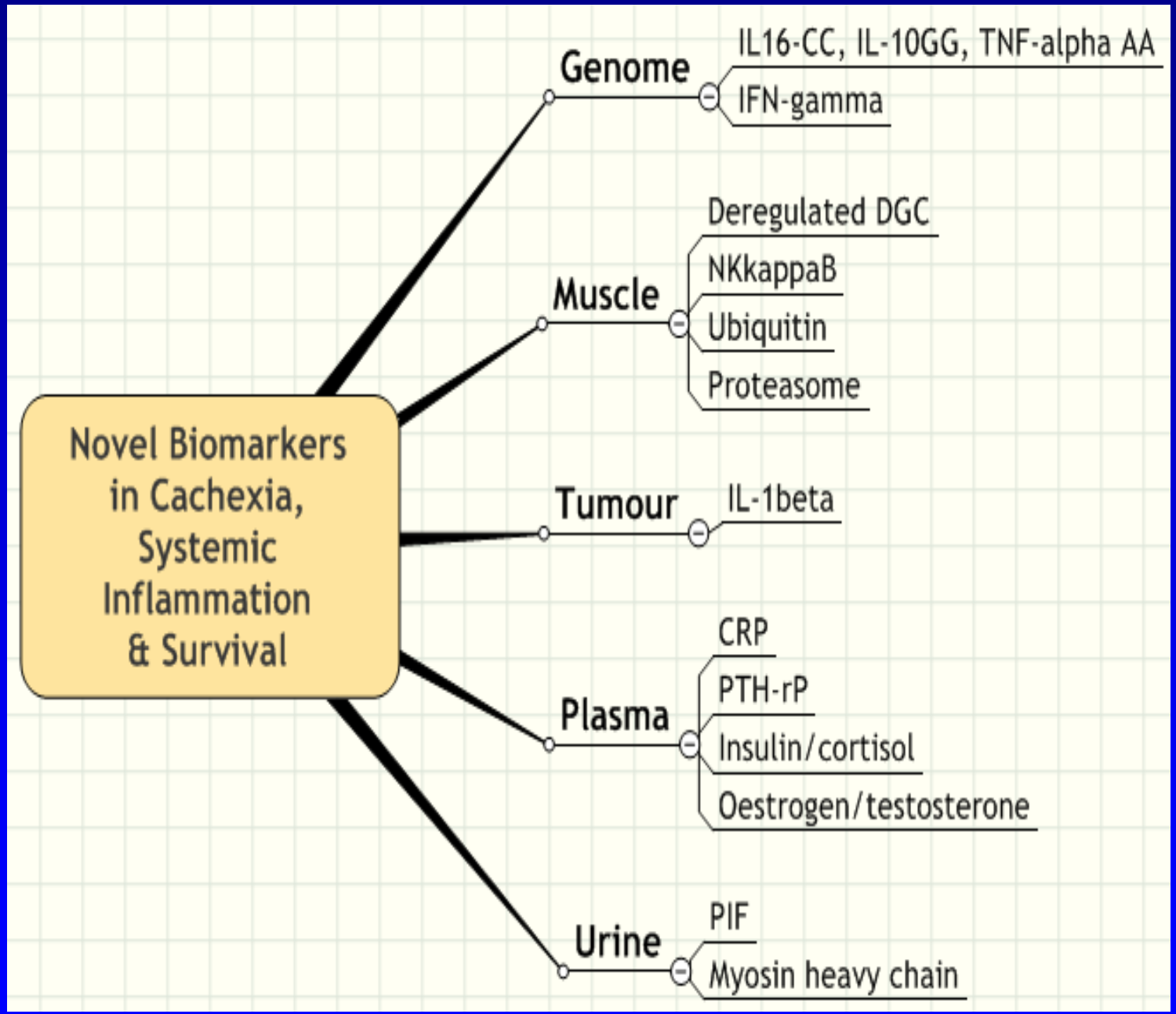
The emerging role of biological-genetic markers to predict the catabolic drive of cancer :EPCRC data and further perspectives

KCH Fearon

Classification of Cachexia: Cachexia represents a spectrum. Not all patients will progress down the spectrum. There are no robust biomarkers to identify those in the pre-cachectic phase who are likely to complete the journey or the rate at which they will do so.



Biomarkers



Ann Surg Oncol 2004, 14, 329-39

Hum Immunol 2004, 65, 1405-8

Cancer Cell 2005, 8, 421-32

In preparation

Oncol Rep 2005, 14, 257-63

Int J Biochem Cell Biol 2005, 37, 2196-06

Br J Cancer 2006, 95, 1568-75

Cancer Res 1988, 48, 2590-5;
Cancer 1995, 75, 2077-82;
Am J Clin Nutr 2006, 83, 1345-50

Cancer 2005, 103, 1810-8

Am J Physiol 2000, 279, E707-14

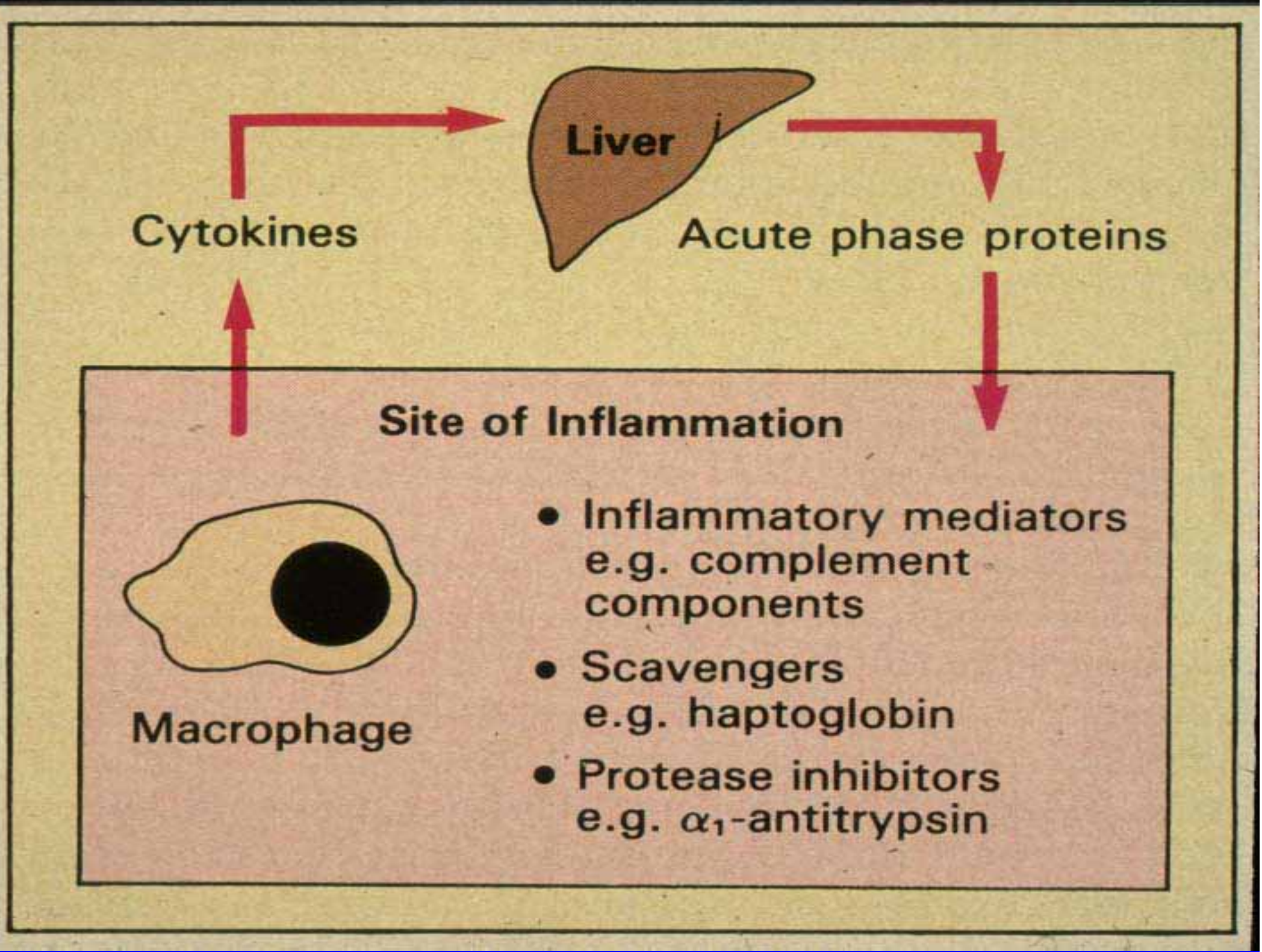
In preparation

Nature 1996, 379, 739-42

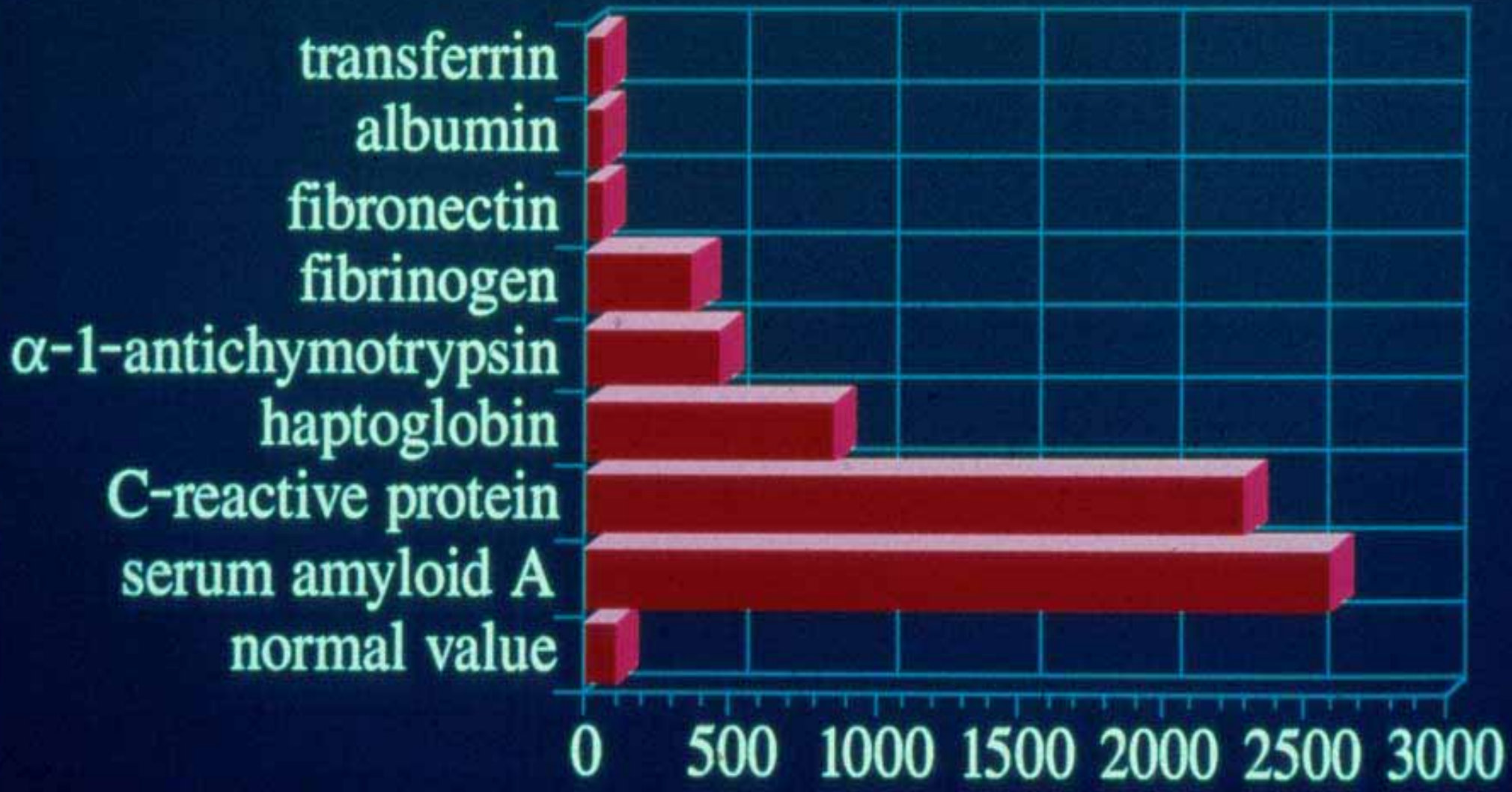
In preparation

CRP

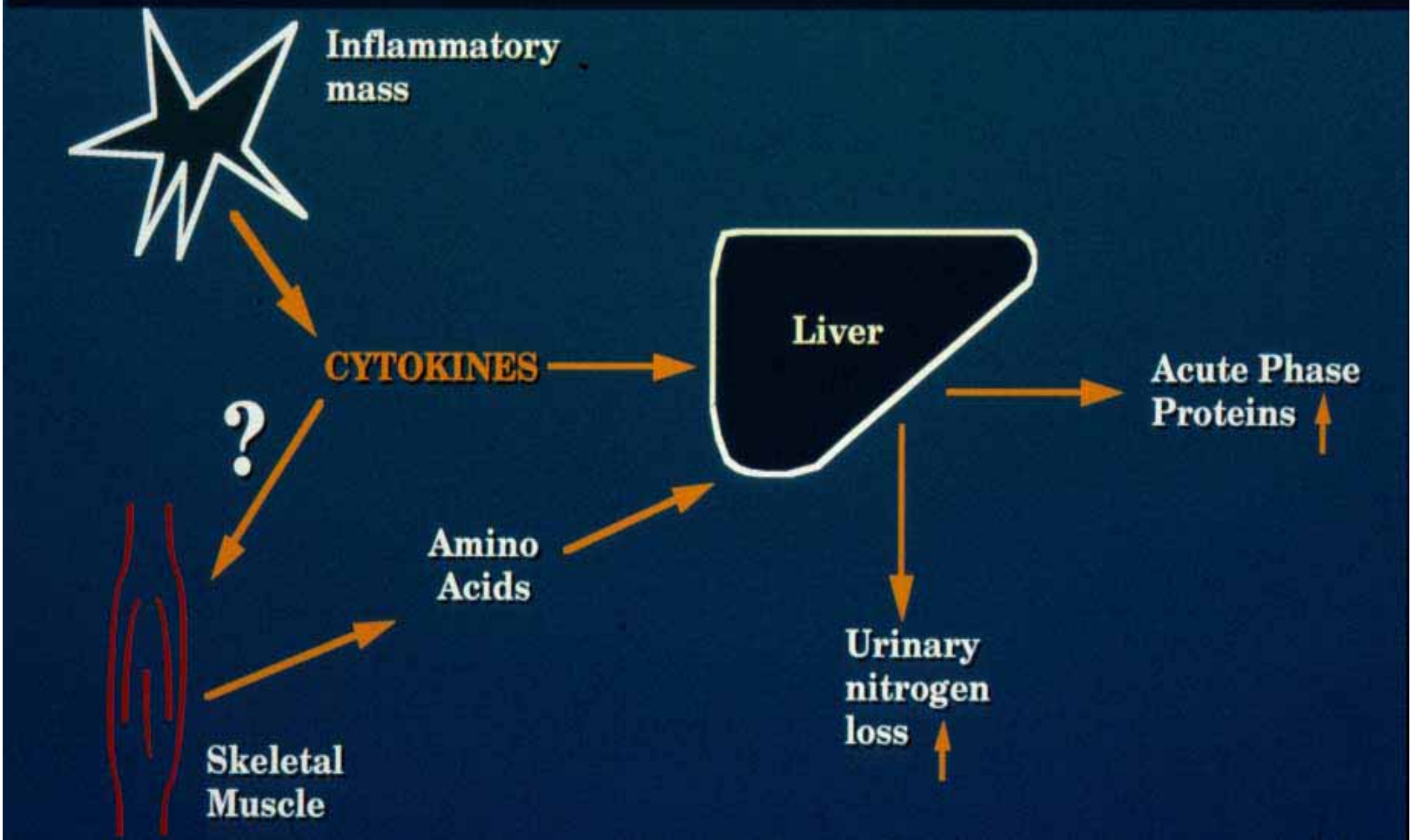
(SYSTEMIC INFLAMMATION)


Liver
Cytokines
Acute phase proteins
Site of Inflammation
Macrophage
• Inflammatory mediators e.g. complement components
• Scavengers e.g. haptoglobin
• Protease inhibitors e.g. α_1-antitrypsin

IL-6 regulates the synthesis of acute phase proteins

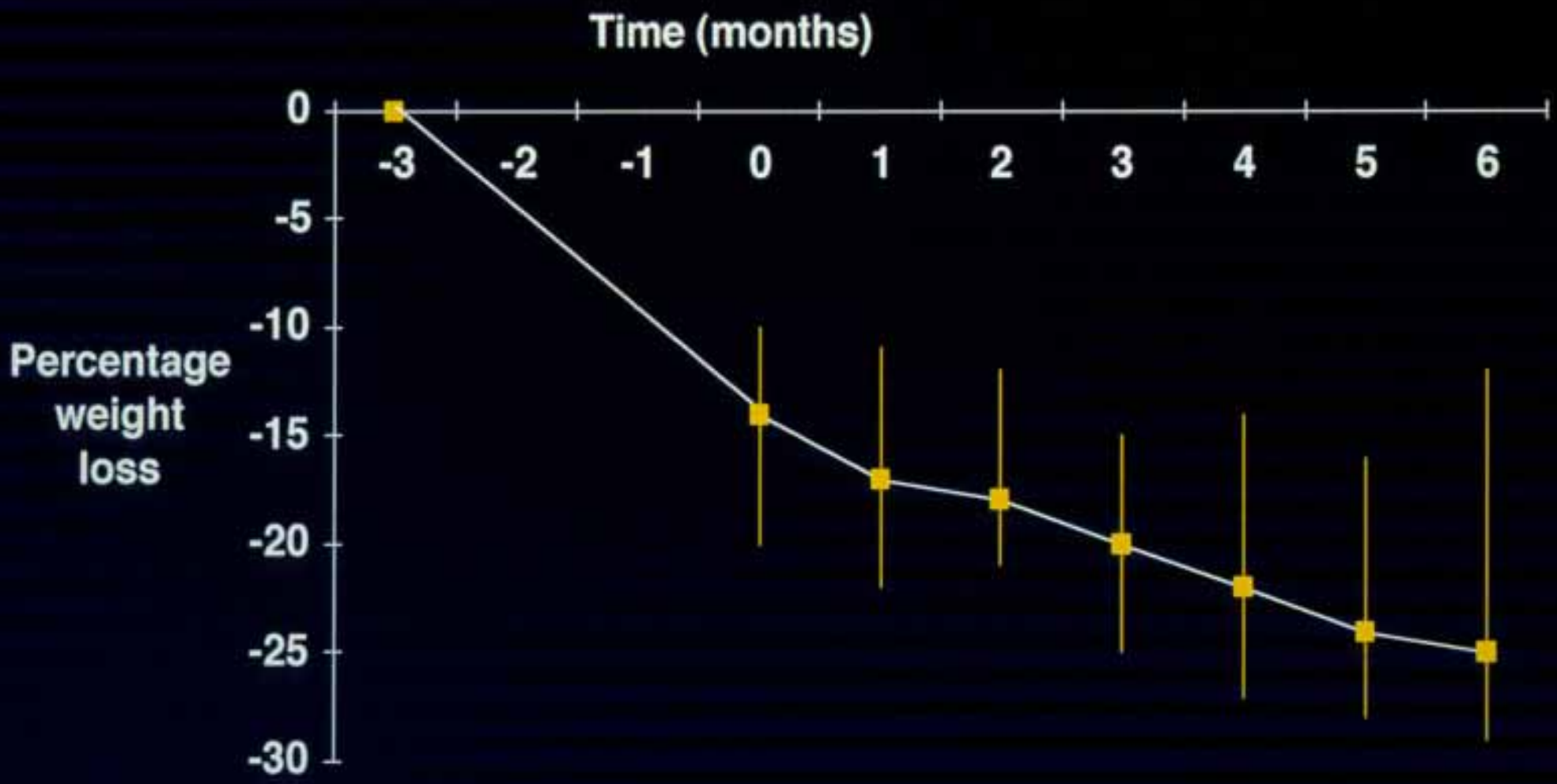


Protein Metabolism During Inflammation



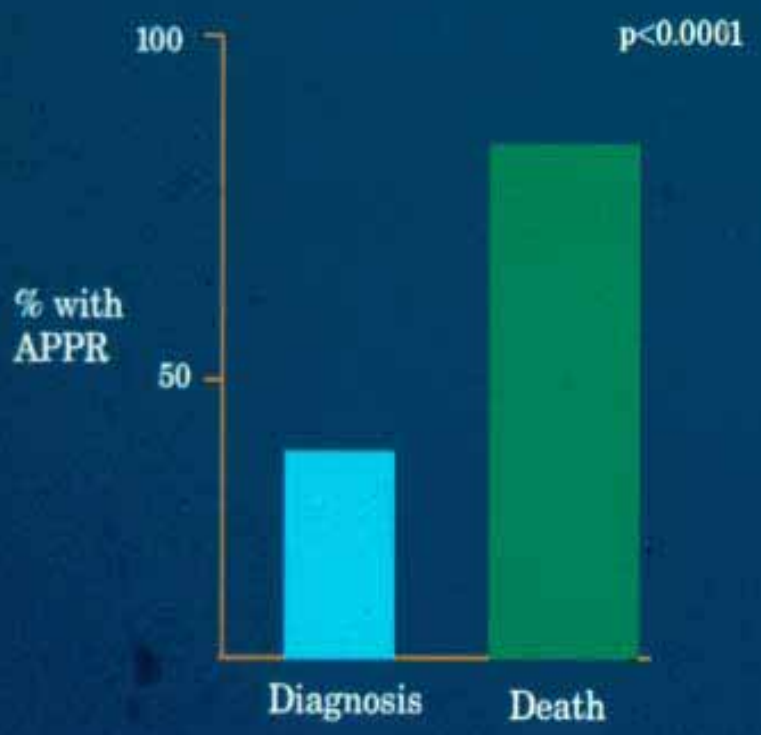
Weight loss in patients with advanced pancreatic cancer (n=20)

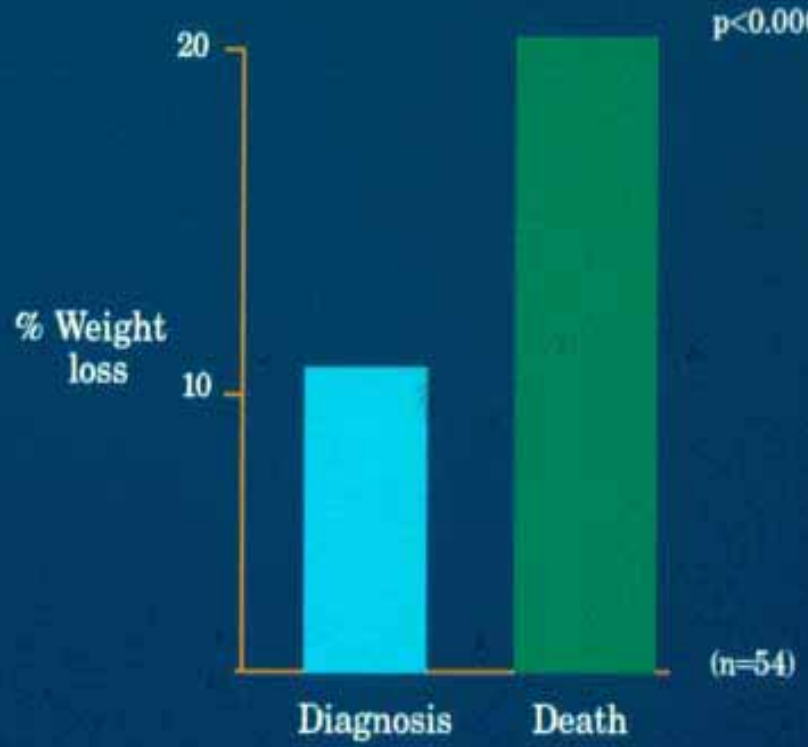
85% of patients cachectic at diagnosis



Wigmore et al, 1997

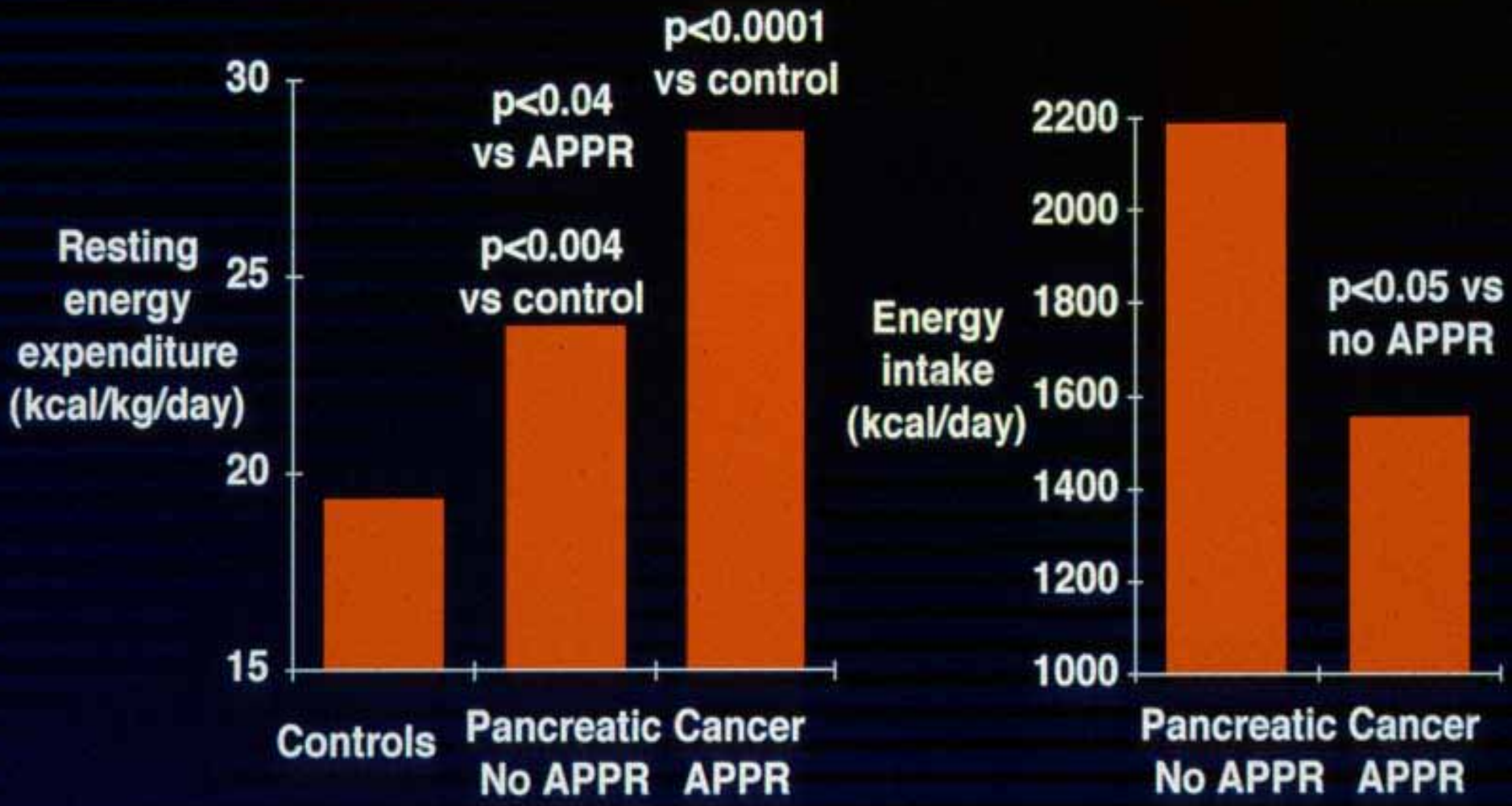
The severity of weight loss and incidence of an acute phase response during disease progression in pancreatic cancer



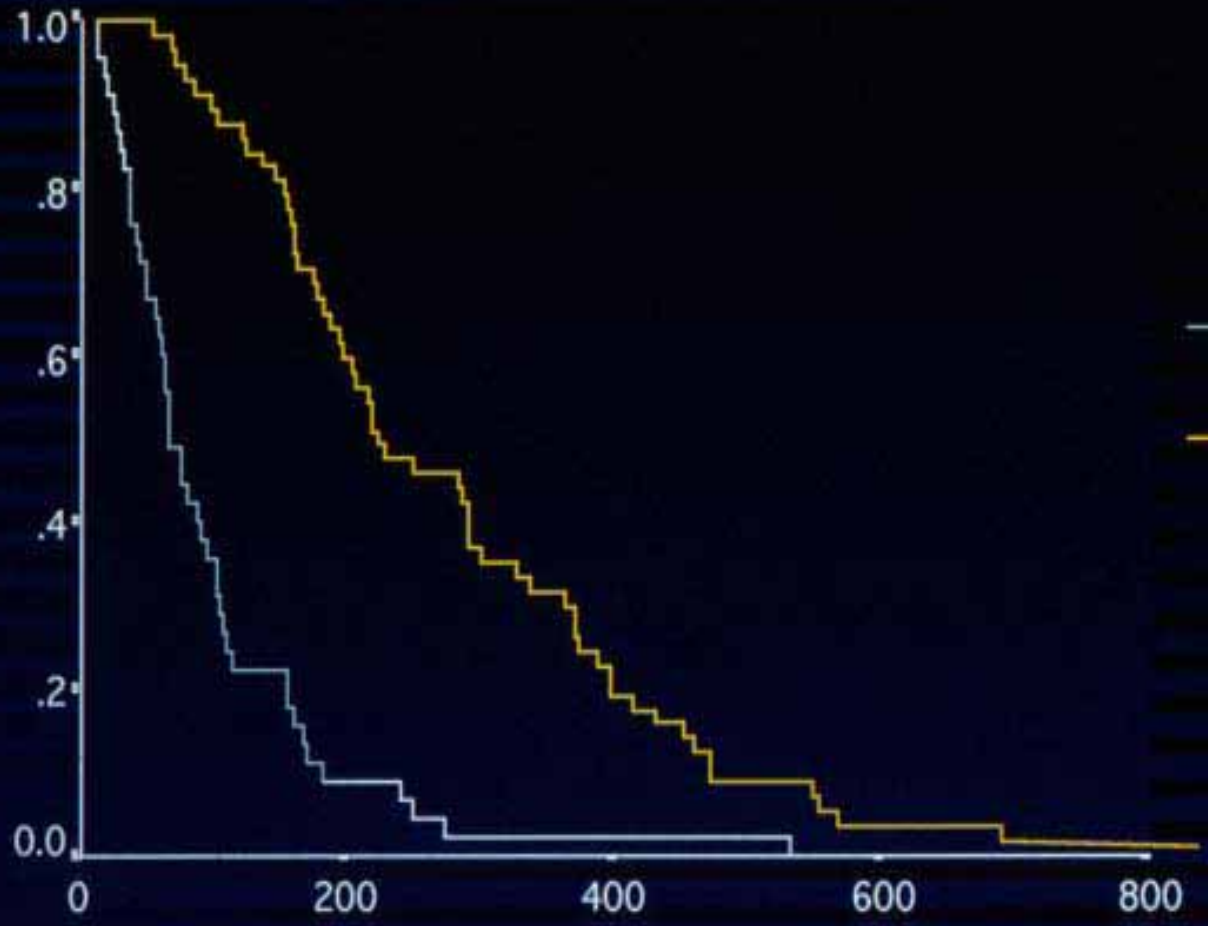


Falconer et al, 1995
Cancer 75; 2077–2082

The effect of the acute phase protein response on energy intake and expenditure in pancreatic cancer

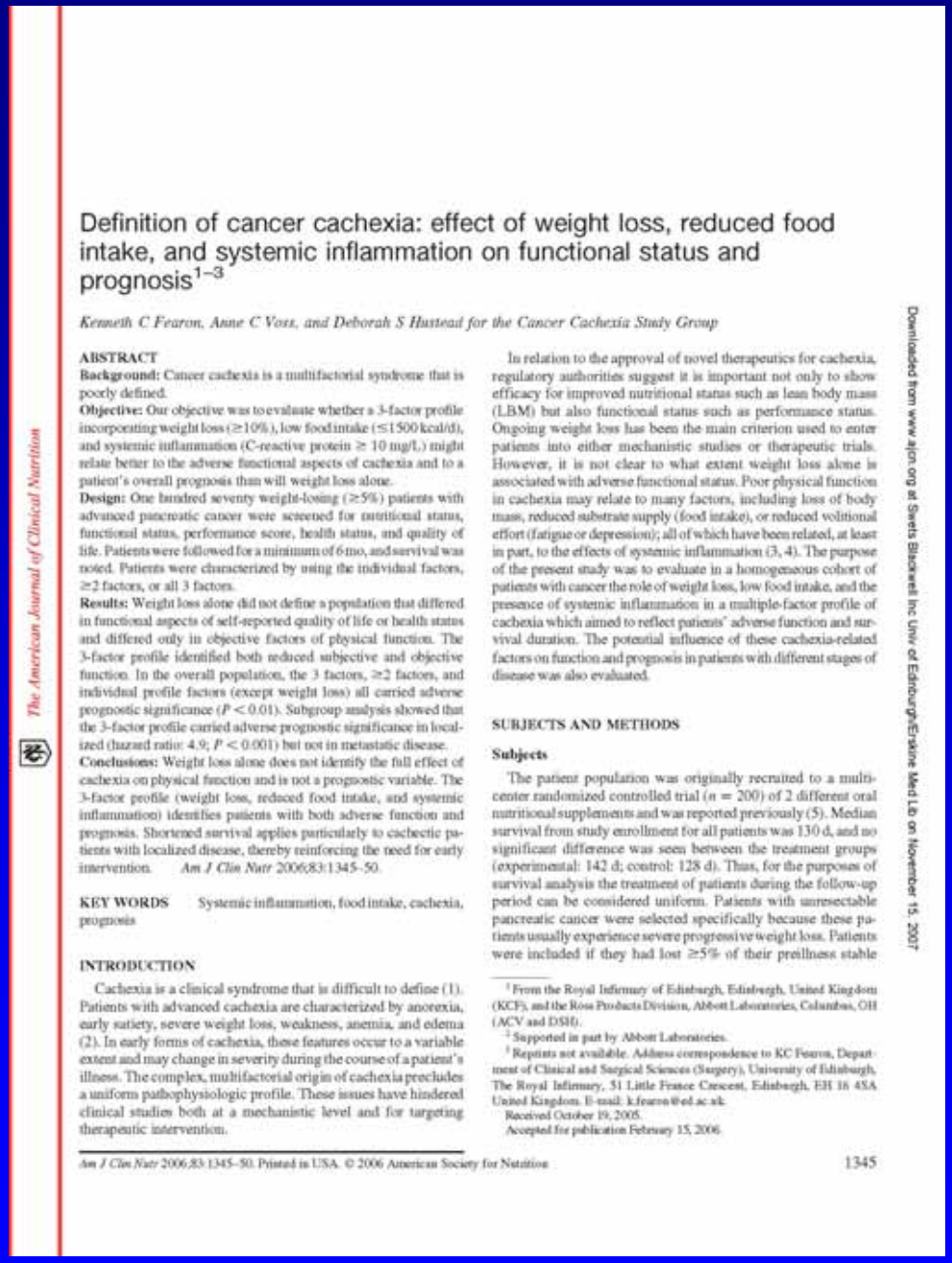


Survival of patients with unresectable pancreatic cancer stratified for APPR



p<0.0001
Cox regression

Falconer et al, 1995



Definition of cancer cachexia: effect of weight loss, reduced food intake, and systemic inflammation on functional status and prognosis[1–3]

Kenneth C Fearon, Anne C Voss, and Deborah S Hustead for the Cancer Cachexia Study Group

The American Journal of Clinical Nutrition

ABSTRACT

Background: Cancer cachexia is a multifactorial syndrome that is poorly defined.

Objective: Our objective was to evaluate whether a 3-factor profile incorporating weight loss (≥10%), low food intake (≤1500 kcal/d), and systemic inflammation (C-reactive protein ≥ 10 mg/L) might relate better to the adverse functional aspects of cachexia and to a patient's overall prognosis than will weight loss alone.

Design: One hundred seventy weight-losing (≥5%) patients with advanced pancreatic cancer were screened for nutritional status, functional status, performance score, health status, and quality of life. Patients were followed for a minimum of 6 mo, and survival was noted. Patients were characterized by using the individual factors, ≥2 factors, or all 3 factors.

Results: Weight loss alone did not define a population that differed in functional aspects of self-reported quality of life or health status and differed only in objective factors of physical function. The 3-factor profile identified both reduced subjective and objective function. In the overall population, the 3 factors, ≥2 factors, and individual profile factors (except weight loss) all carried adverse prognostic significance ($P < 0.01$). Subgroup analysis showed that the 3-factor profile carried adverse prognostic significance in localized (hazard ratio: 4.9; $P < 0.001$) but not in metastatic disease.

Conclusions: Weight loss alone does not identify the full effect of cachexia on physical function and is not a prognostic variable. The 3-factor profile (weight loss, reduced food intake, and systemic inflammation) identifies patients with both adverse function and prognosis. Shortened survival applies particularly to cachectic patients with localized disease, thereby reinforcing the need for early intervention. *Am J Clin Nutr* 2006;83:1345–50.

KEY WORDS Systemic inflammation, food intake, cachexia, prognosis

INTRODUCTION

Cachexia is a clinical syndrome that is difficult to define (1). Patients with advanced cachexia are characterized by anorexia, early satiety, severe weight loss, weakness, anemia, and edema (2). In early forms of cachexia, these features occur to a variable extent and may change in severity during the course of a patient's illness. The complex, multifactorial origin of cachexia precludes a uniform pathophysiologic profile. These issues have hindered clinical studies both at a mechanistic level and for targeting therapeutic intervention.

In relation to the approval of novel therapeutics for cachexia, regulatory authorities suggest it is important not only to show efficacy for improved nutritional status such as lean body mass (LBM) but also functional status such as performance status. Ongoing weight loss has been the main criterion used to enter patients into either mechanistic studies or therapeutic trials. However, it is not clear to what extent weight loss alone is associated with adverse functional status. Poor physical function in cachexia may relate to many factors, including loss of body mass, reduced substrate supply (food intake), or reduced volitional effort (fatigue or depression); all of which have been related, at least in part, to the effects of systemic inflammation (3, 4). The purpose of the present study was to evaluate in a homogeneous cohort of patients with cancer the role of weight loss, low food intake, and the presence of systemic inflammation in a multiple-factor profile of cachexia which aimed to reflect patients' adverse function and survival duration. The potential influence of these cachexia-related factors on function and prognosis in patients with different stages of disease was also evaluated.

SUBJECTS AND METHODS

Subjects

The patient population was originally recruited to a multicenter randomized controlled trial ($n = 200$) of 2 different oral nutritional supplements and was reported previously (5). Median survival from study enrollment for all patients was 130 d, and no significant difference was seen between the treatment groups (experimental: 142 d; control: 128 d). Thus, for the purposes of survival analysis the treatment of patients during the follow-up period can be considered uniform. Patients with unresectable pancreatic cancer were selected specifically because these patients usually experience severe progressive weight loss. Patients were included if they had lost ≥5% of their preillness stable

[1] From the Royal Infirmary of Edinburgh, Edinburgh, United Kingdom (KCF), and the Ross Products Division, Abbott Laboratories, Columbus, OH (ACV and DSH).

[2] Supported in part by Abbott Laboratories.

[3] Reprints not available. Address correspondence to KC Fearon, Department of Clinical and Surgical Sciences (Surgery), University of Edinburgh, The Royal Infirmary, 51 Little France Crescent, Edinburgh, EH 16 4SA United Kingdom. E-mail: k.fearon@ed.ac.uk.

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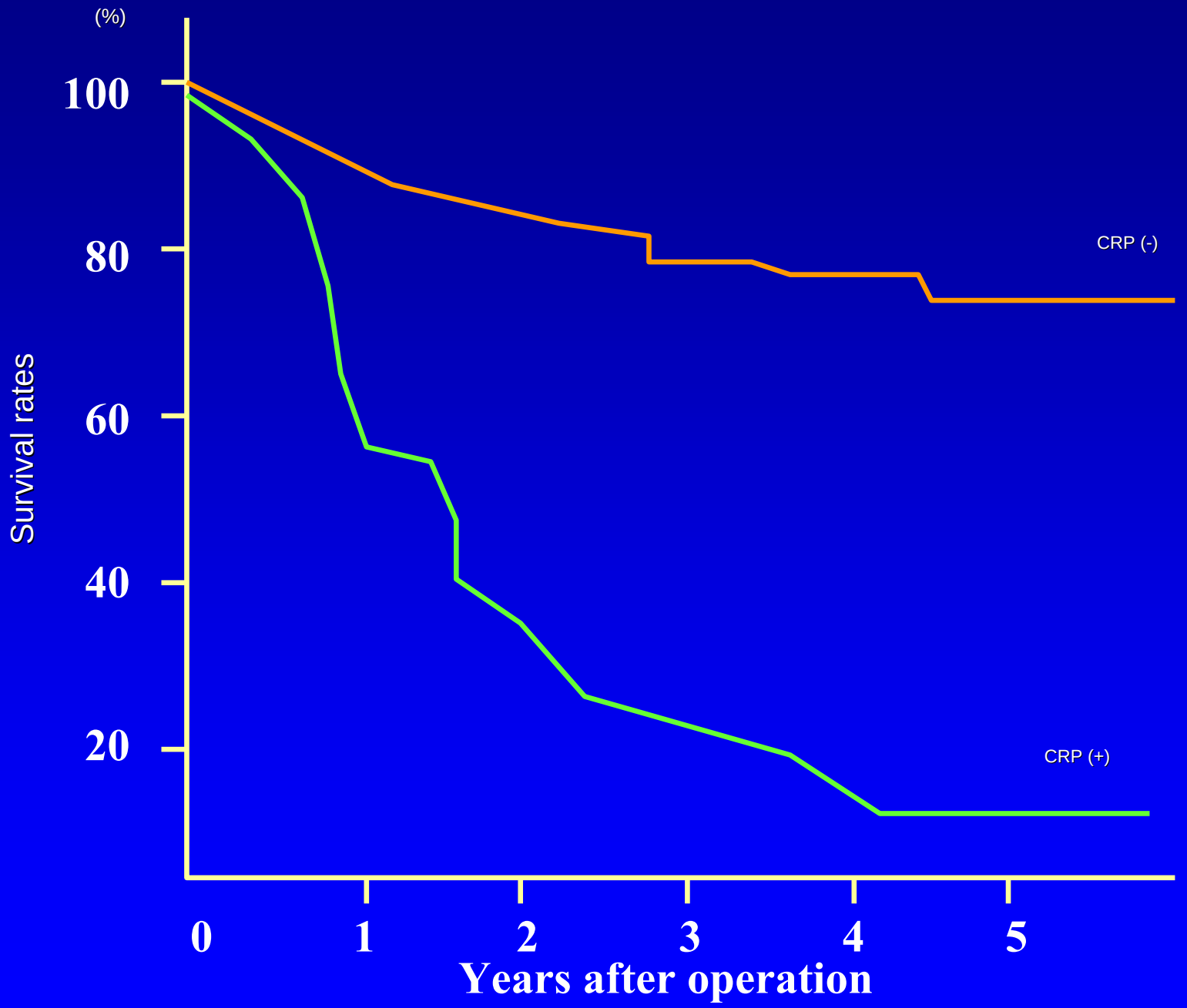
Cachexia =

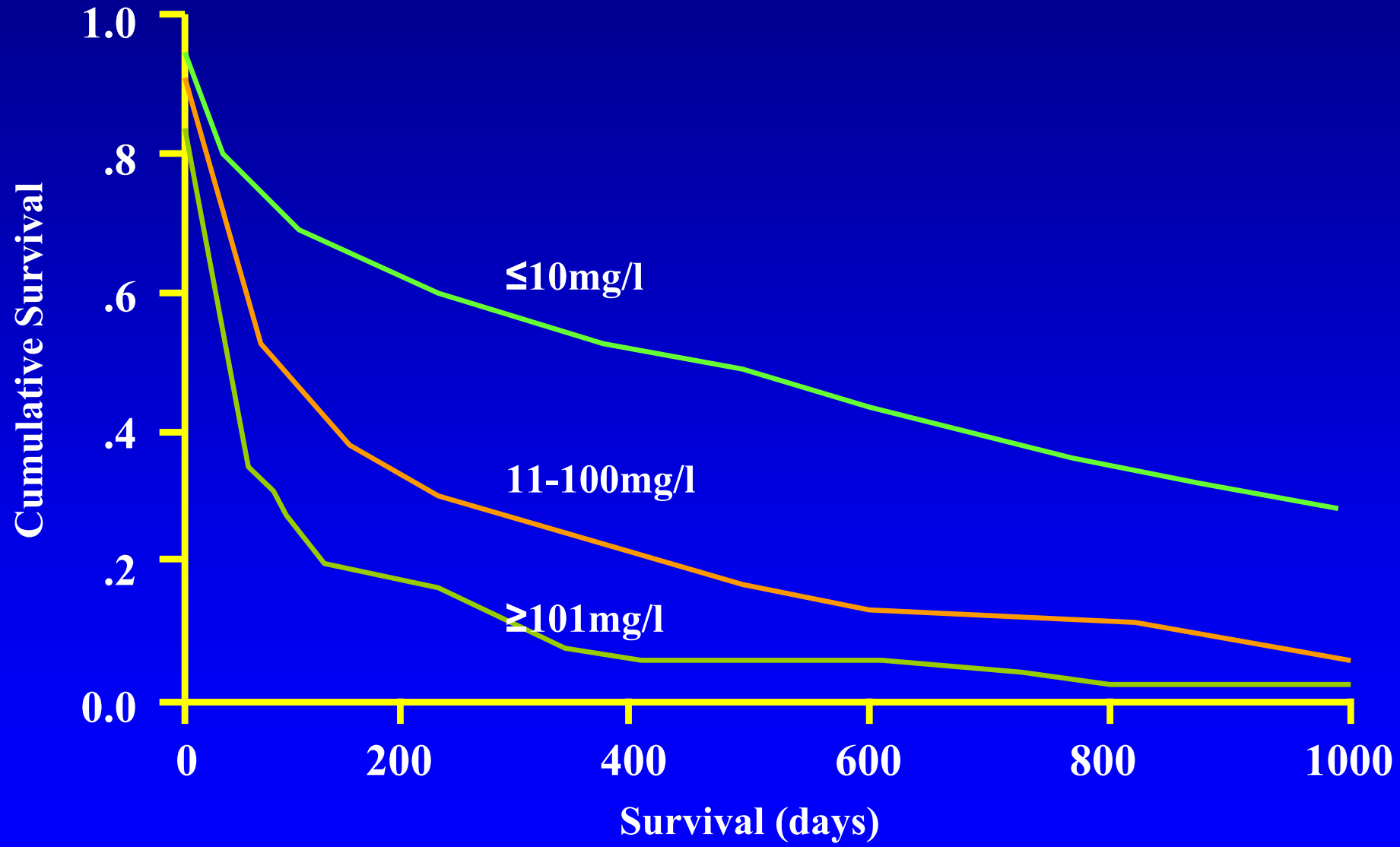
- Weight loss > 10%
- CRP > 10 mg/L
- Food intake < 1500 kcal

Survival following oesophageal resection (n=262)



Nozoe et al, Am. J. Surg. 2001, 182; 197-201

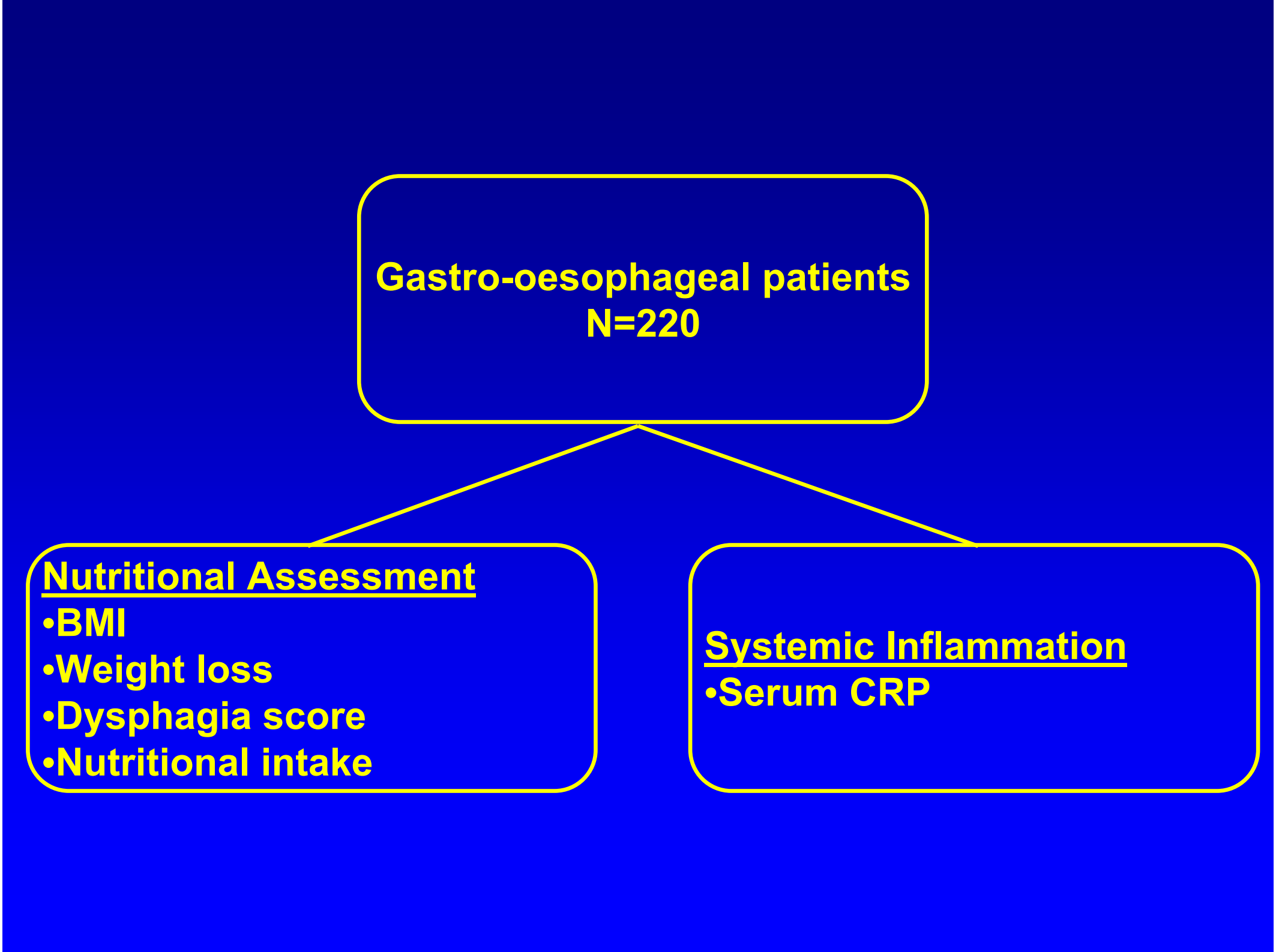
Cancer Specific Survival in Advanced Cancer (n=772)



McMillan et al, Nutrition and Cancer, 2001 ,41; 64-51

Which APPR best predicts weight loss?

Can we ascribe a relative importance to systemic inflammation in the genesis of cachexia?


Gastro-oesophageal patients
N=220
Nutritional Assessment
•BMI
•Weight loss
•Dysphagia score
•Nutritional intake
Systemic Inflammation
•Serum CRP

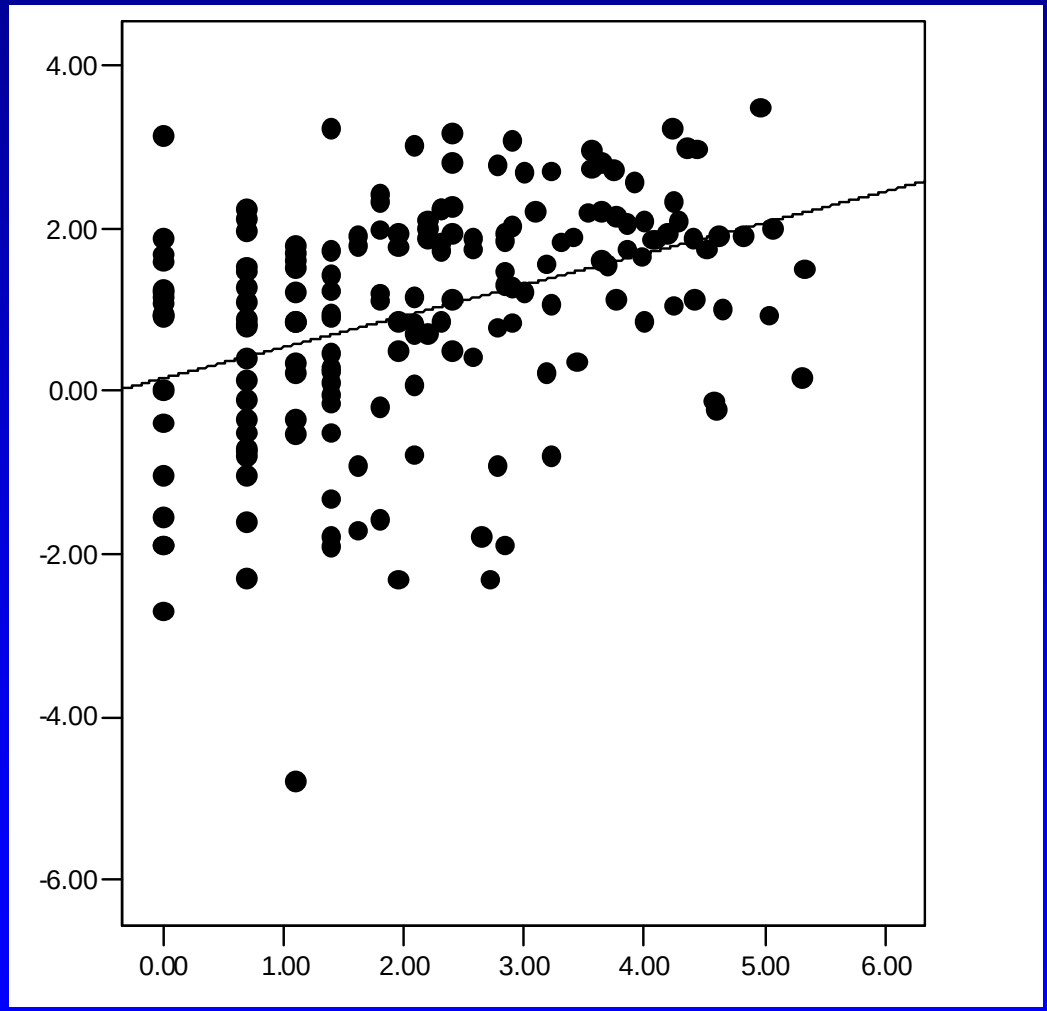
Relationship between serum concentrations of acute phase proteins with markers of nutritional status at the time of diagnosis of OG cancer (n=220).

	Wt loss		Rate of wt loss	
	r	P	r	P
CRP	0.30	<0.001	0.36	<0.001
ACT	0.23	0.001	0.27	<0.001
Haptoglobin	0.15	0.038	0.16	0.020
Albumin	-0.31	<0.001	-0.35	<0.001
Transferrin	-0.30	<0.001	-0.26	<0.001

Spearman rank analysis; r = correlation coefficient

Deans et al (submitted)

Relationship between serum CRP and rate of weight loss in newly diagnoses patients with OG cancer (n=220)



$p < 0.001$

$r = 0.36$

Multiple regression analysis of variables associated with increased weight loss in newly diagnosed patients with OG cancer (n=220)

	Regression coefficient	95% confidence interval	F-test	Estimates of effect size (%)*	P value
Dietary intake	0.28	1.80 to 5.09	1.9	38	<0.001
Stage of disease	0.17	0.31 to 2.62	0.9	28	0.013
CRP conc (ln)	0.17	0.18 to 1.77	1.3	34	0.017

*multivariate general linear model

Deans et al (submitted)

In patients with OG cancer:

- **Systemic inflammation** is of similar importance to reduced food intake or mechanical obstruction by the tumour in the genesis of weight loss

Serum CRP level: a complex trait!

Environmental

- Age ↑
- Sex (f) ↑
- Smoking ↑
- BMI (O) ↑
- HRT ↑
- Statin ↓

Heritable

- 27 – 40% genetically determined
- 1 – 2% determined by SNP's within CRP gene

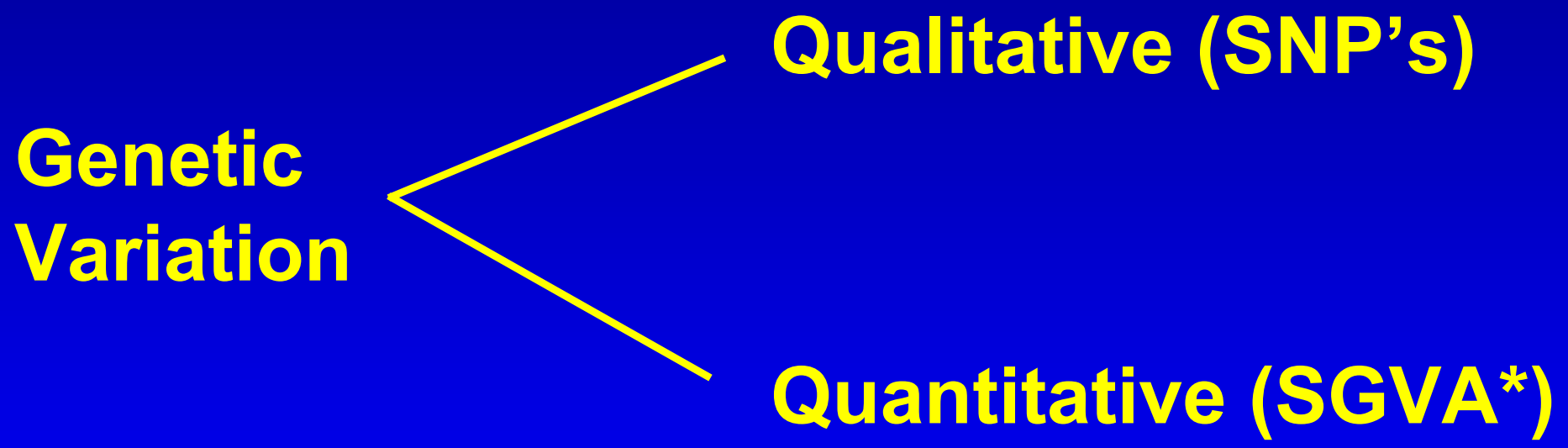
Ledue TB Clin Chem 2003, 49, 1258-71
Dupuis J Atherosclerosis 2005, 182, 307-14
Kathuicstan S Circulation 2006, 113, 1415-23

Genetic Biomarkers of Cachexia?

Cachexia in Cancer

- Nutritional insufficiency
- Hypermetabolism
- Inflammation
- Neurohormonal change
- Tumour factors
- Inactivity
- Oxidative stress
- Anabolic hormone insufficiency

Acting on Genetic Predisposition

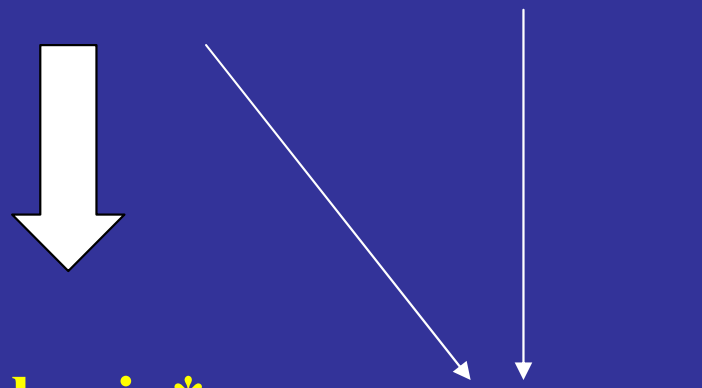


*structured genomic variant architecture

Korbel et al. Science 2007, 318, 420-6

Advanced gastric cancer (n = 214)

Il-1ß + 3594cc | **Il-1ß – 511cc** | **Il-1ß –311cc** | **Il-IRN VNTR**



Cachexia*
(RR: 2.5)

PBMC Il-1↑

(*Weight loss > 10% in previous 6/12)

Population at risk = 17%

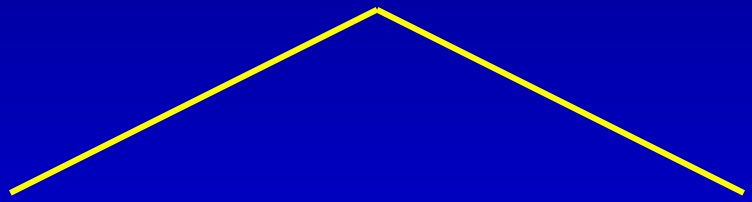
Zhang D, et al 2007
BMC Cancer, 7, 45

Interleukin-10 polymorphisms are independently associated with risk of cachexia among patients with gastro-oesophageal cancer

Deans DAC[1], Tan B[1], Rose-Zerilli M[2], Wigmore SJ[1], Ross JA[1], Howell M[3], Grimble R[4], Fearon KCH[1]

[1]Cell Injury and Apoptosis Section, Tissue Injury and Repair Group, MRC Centre for Inflammation Research, Department of Clinical and Surgical Sciences, Medical School, Edinburgh University, EH8 9AG, UK [2]Histocompatibility and Immunogenetics Laboratory, Human Genetics Division, University of Southampton, UK [3]Department of Histocompatibility and Immunogenetics, National Blood Service, Holland Drive, Newcastle-upon-Tyne, NE2 4NQ, UK [4]Institute of Human Nutrition, School of Medicine, University of Southampton, SO16 7PX, UK

Gastro-oesophageal cancer patients (n=203)



Phenotyping

BMI
Anthropometry
Dysphagia score
Dietary intake
Systemic inflammation (CRP)

Genotyping

TaqMan allelic discrimination

- IL-1 – S11
- IL-6 – 174
- IL-10 – 1082
- TNFα – 308
- LT α +252

} Cytokine polymorphisms

Cytokine	Allele	Study patients n (%)
IL-1β -511	CC	89 (45.4)
	CT	81 (41.3)
	TT	26 (13.3)
	HWE‡	0.27
IL-6 –174	GG	71 (36.0)
	GC	83 (42.1)
	CC	43 (21.8)
	HWE	0.05
IL-10 1082	GG	54 (27.0)
	AG	93 (46.5)
	AA	53 (26.5)
	HWE	0.32
TNFα -308	GG	124 (62.0)
	AG	61 (30.5)
	AA	15 (7.5)
	HWE	0.06
LTα +252	AA	82 (42.1)
	AG	84 (43.1)
	GG	29 (14.9)
	HWE	0.33

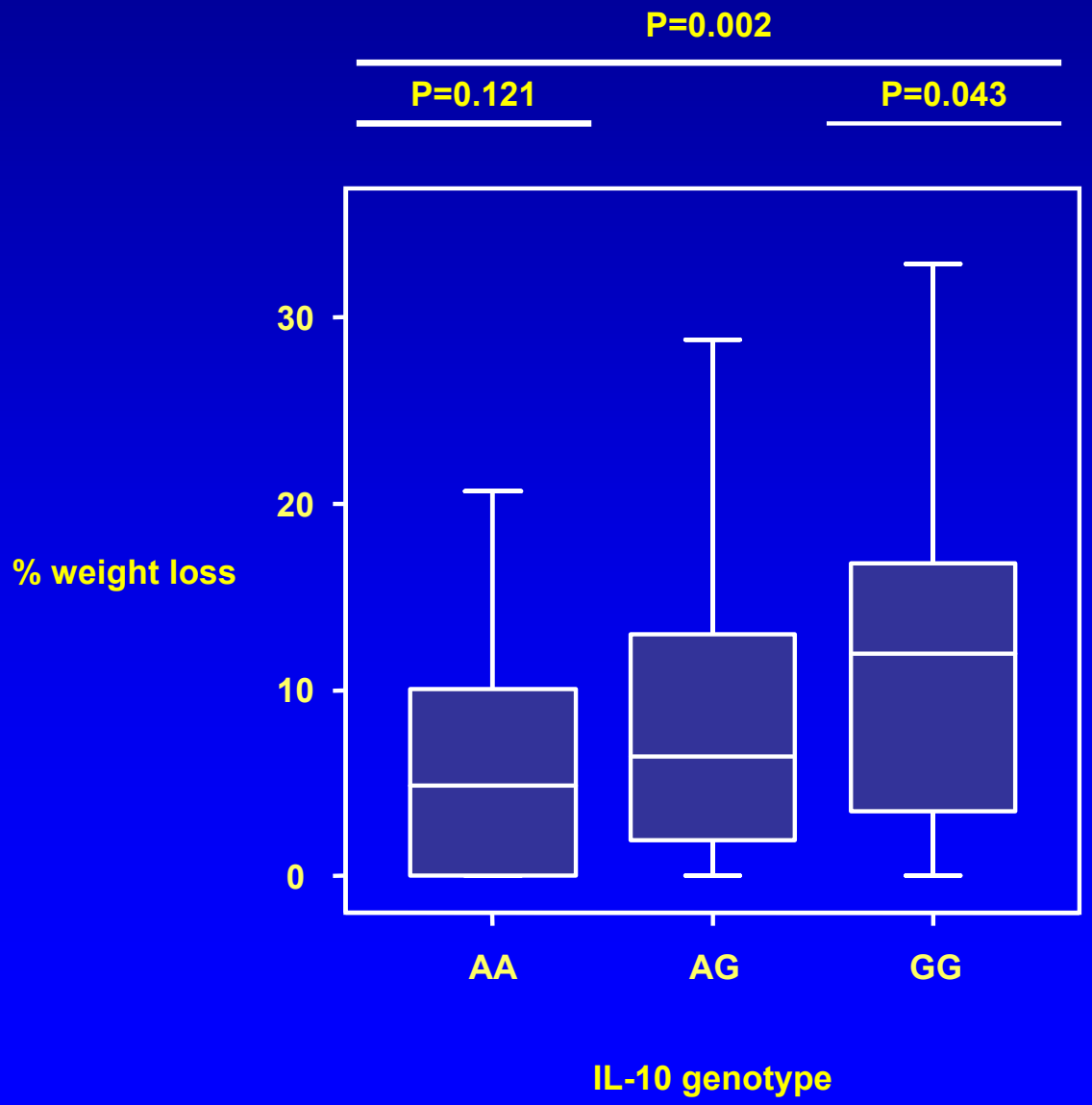
‡P value for Hardy-Weinberg Equilibrium (HWE) (Chi-squared test)

Nutritional variables for the patient group measured at the time of diagnosis stratified by IL-10 genotype.

	IL-10 Genotype			P value
	AA (n=53)	AG (n=93)	GG (n=54)	
Pre-illness BMI	26.6 (23.1-30.6)	26.4 (23.9-30.1)	26.3 (24.4-30.2)	0.959
BMI at diagnosis	25.6 (21.7-28.7)	24.6 (21.2-27.9)	23.9 (20.7-27.4)	0.214
Total body weight loss (%)	4.9 (0-10.2)	7.1 (1.1-13.9)	12.0 (3.3-16.8)	0.007
Rate of weight loss (% per month)	2.2 (0-4.6)	3.2 (0.5-6.3)	5.4 (1.5-7.6)	0.008
Mid-arm circumference (percentile group)	10 (5-25)	10 (1-25)	10 (1-25)	0.347
Triceps skinfold thickness (percentile group)	25 (25-50)	25 (10-50)	25 (5-50)	0.186
Arm-muscle circumference (percentile group)	5 (1-25)	5 (1-25)	10 (1-50)	0.748
Food diary intake¶ Energy kcal (% of EAR) Protein (% of RNI)	65 (56-91) 115 (104-192)	87 (68-93) 142 (109-170)	82 (64-104) 129 (97-169)	 0.116 0.325
Dietary intake Normal Reduced Poor/minimal	23 [44] 21 [40] 9 [16]	35 [38] 44 [47] 14 [15]	13 [24] 32 [59] 9 [18]	0.123*
Dysphagia score 0 1 2 3 4	22 [42] 15 [28] 11[20] 5 [10] 0	39 [42] 19 [21] 19 [21] 14 [15] 2 [2]	24 [45] 6 [12] 10 [18] 13 [24] 1 [2]	0.236*

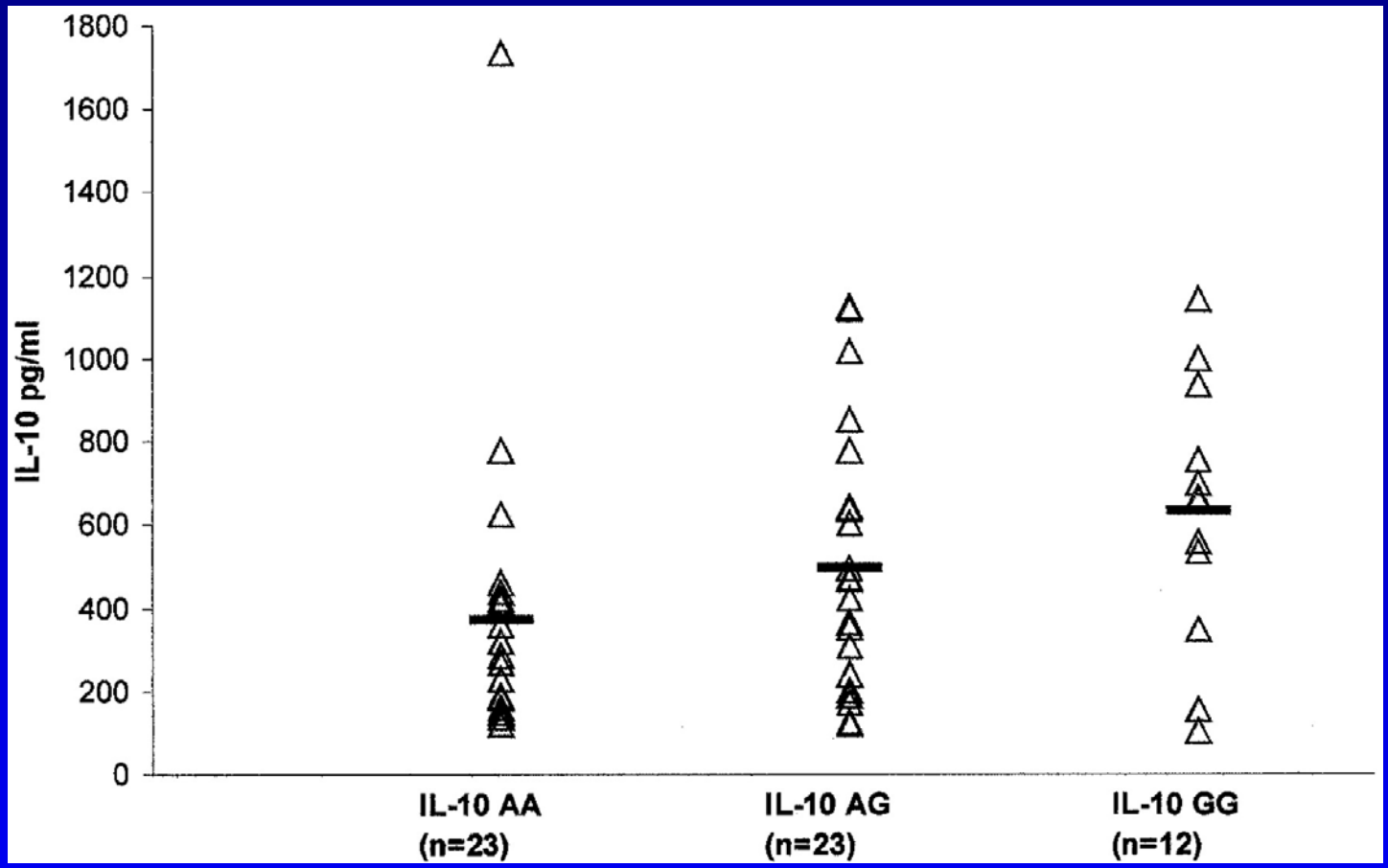
¶ Calculated from a subgroup of 22 patients. EAR = estimated average requirement. RNI = reference nutritional intake. Values are median (inter-quartile range). [%]. Kruskal-Wallis Test except *Chi-square test.

The IL-10 genotype is associated with increased total weight loss at the time of diagnosis



FUNCTIONAL SIGNIFICANCE?

IL-10 -1082 genotype and IL-10 production



Lipopolysaccharide-stimulated whole-blood IL-10 concentrations (*levels* indicate mean) in 58 patients with pneumococcal disease according to IL-10–1082 genotype. IL-10 concentrations in IL-10 GG patients versus IL-10 A/G or IL-10 A/A patients (p = 0.04).

Schaaf BM et al. Am J Respir Crit Care Med 2003 168, 476-480

Association between genotype INTERLEUKIN-10–1082 GG and sepsis severity tested with the Cochrane–Armitage trend test

	Interleukin-10–1082						
	AA/AG		GG				
	n	%	n	%	OR	95% CI	Corrected p
Nonsepsis	16	30.19	2	12.50			
Sepsis	23	43.40	5	31.25	2.065	1.156–3.870	
Severe sepsis	8	15.09	2	12.50	4.264	1.337–14.977	
Septic shock	6	11.32	7	43.75	8.805	1.545–57.962	0.027

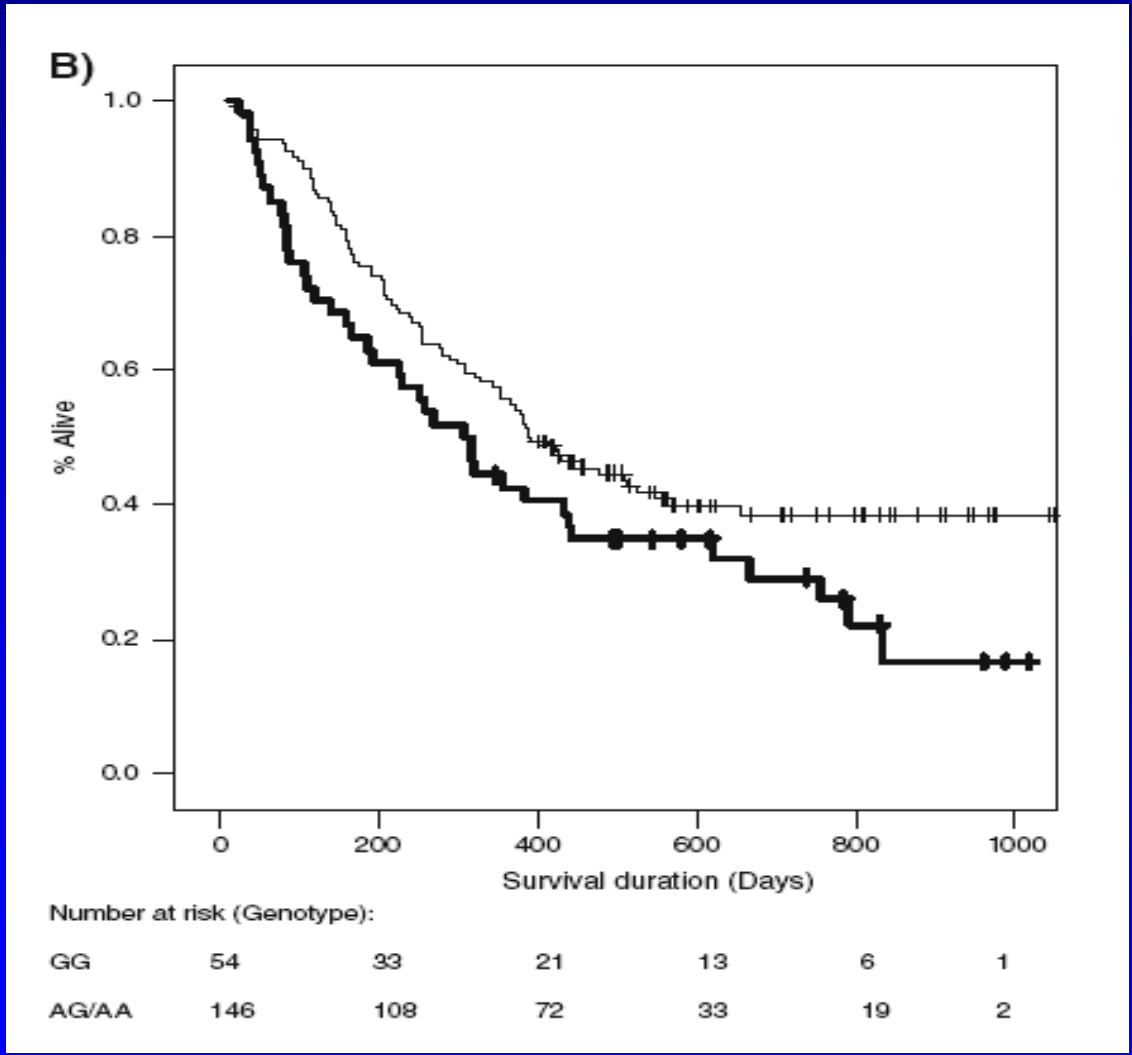
Definition of abbreviations: CI = confidence interval; OR = odds ratio.

IL-10 GG is associated with increasing sepsis severity, most prominent for septic shock.

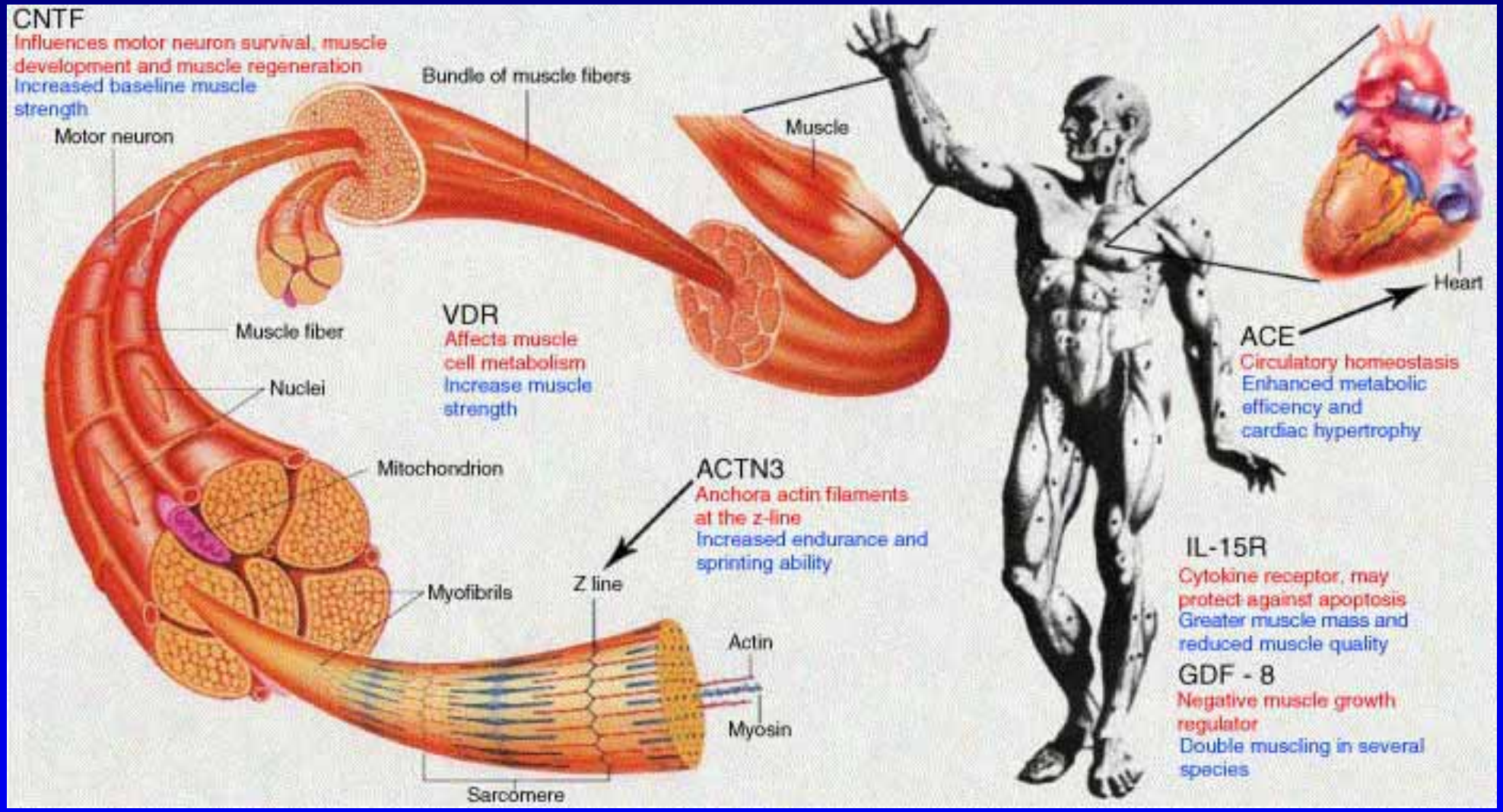
The p value is corrected for testing multiple polymorphisms (times three).

Schaaf BM et al. Am J Respir Crit Care Med 2003 168, 476-480

Survival of Gastro-oesophageal cancer patients according to IL-10-1082 genotype



Deans C et al 2007 Ann Surg Oncol 14, 329-39



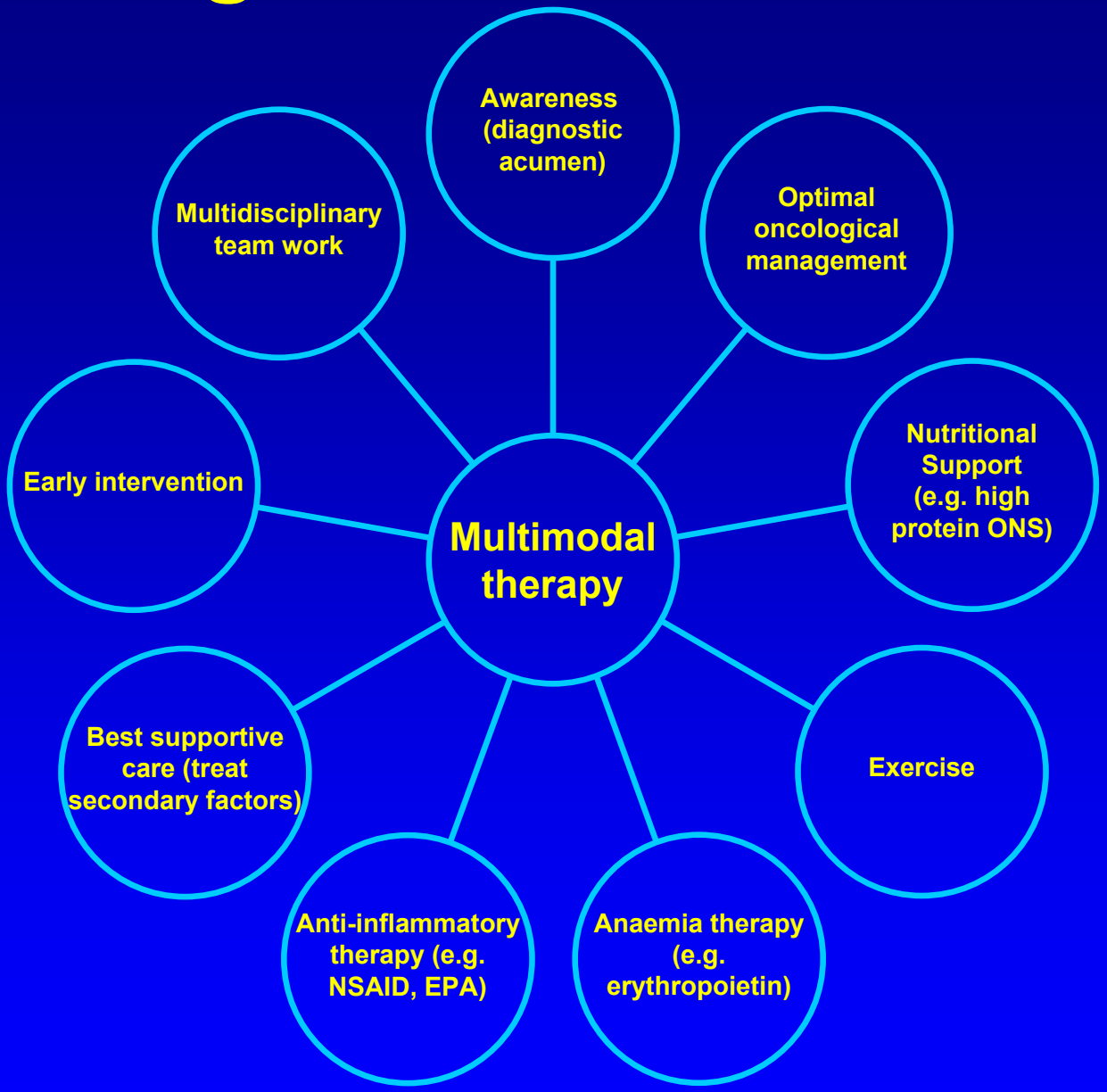
Single nucleotide polymorphisms associated with muscle phenotypes. Function (red) of single nucleotide polymorphisms and published associations (blue) with muscle phenotypes. ACE (angiotensin 1 converting enzyme), GDF-8 (myostatin), ACTN3 (alpha actinin 3), IL-15R (interleukin-15 receptor), CNTF (ciliary neurotrophic factor), VDR (vitamin D receptor).

Gordon ES et al. Int J Biochem Cell Biol. 2005 37, 2064-74

SUMMARY

- Both phenotype (CRP) and genotype (IL-10) aspects of the systemic inflammatory response are strong candidate biomarkers for cancer cachexia
- These and other biomarkers will be explored further by the EPCRC

Pharmacogenetics: cancer cachexia



Multimodal rehabilitation for cancer cachexia. Stabilisation of weight and physical performance are reasonable goals which may be exceeded in some and unmet in others.

Response to exercise training is heterogeneous

- Genomic factors may predict a proportion of non-responders (up to 45% may be neutral or negative responders)

- Functional genomics (gene network activation) may provide a more integrated signal to allow prescription of tailored exercise therapy to maximise the benefits to those who can benefit .

Roth S M J Appl Physiol 2008,104,1243-5
Timmons J J Appl Physiol 2008,104,1250

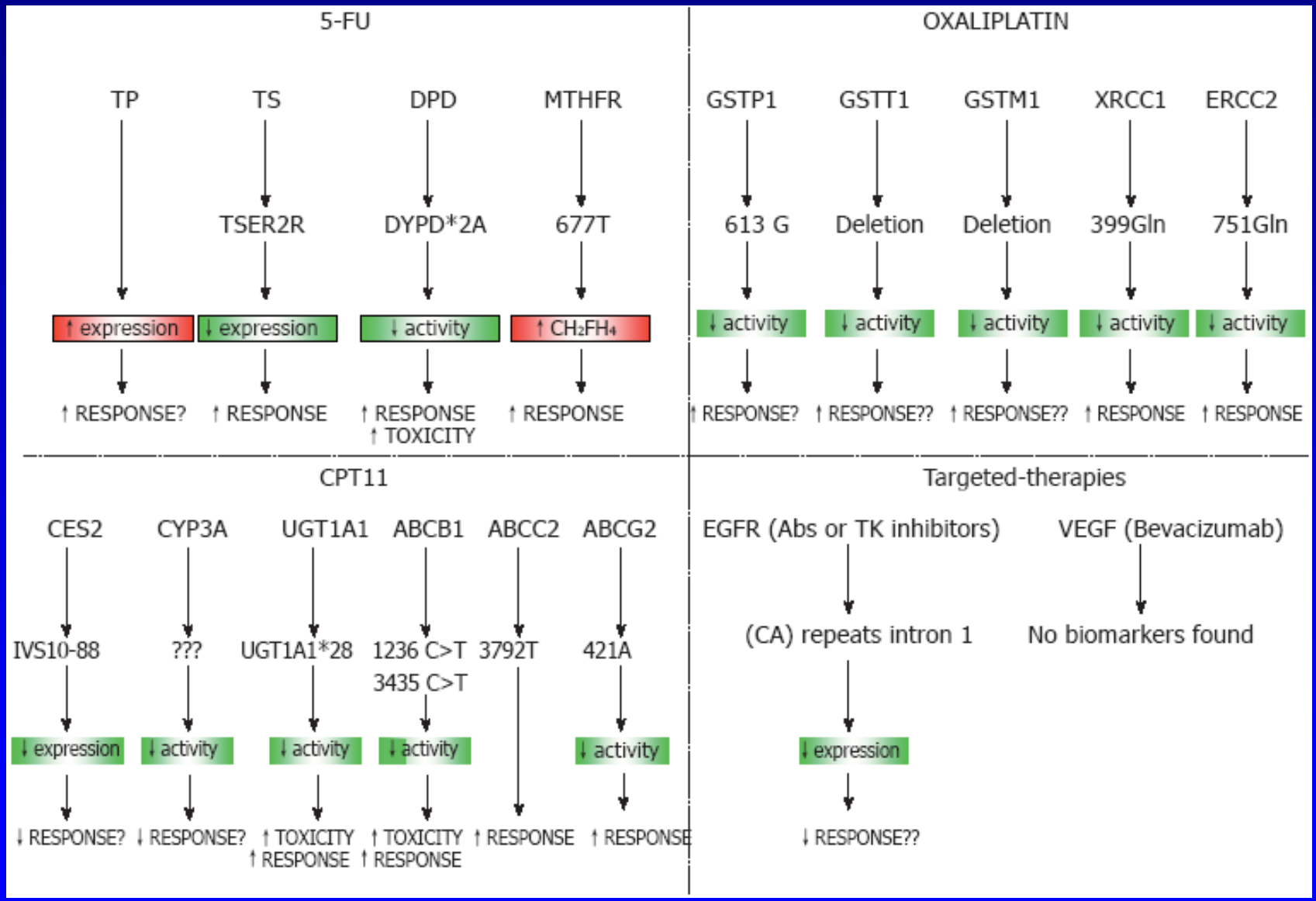
Personal Genome Sequencing: so revealing but little revealed!

James Watson[1]
J Craig Venter[2]

- Statistical likelihoods
- Relevance hard to decipher
- Need a dictionary of genotype-phenotype relationships (e.g. dbGap)

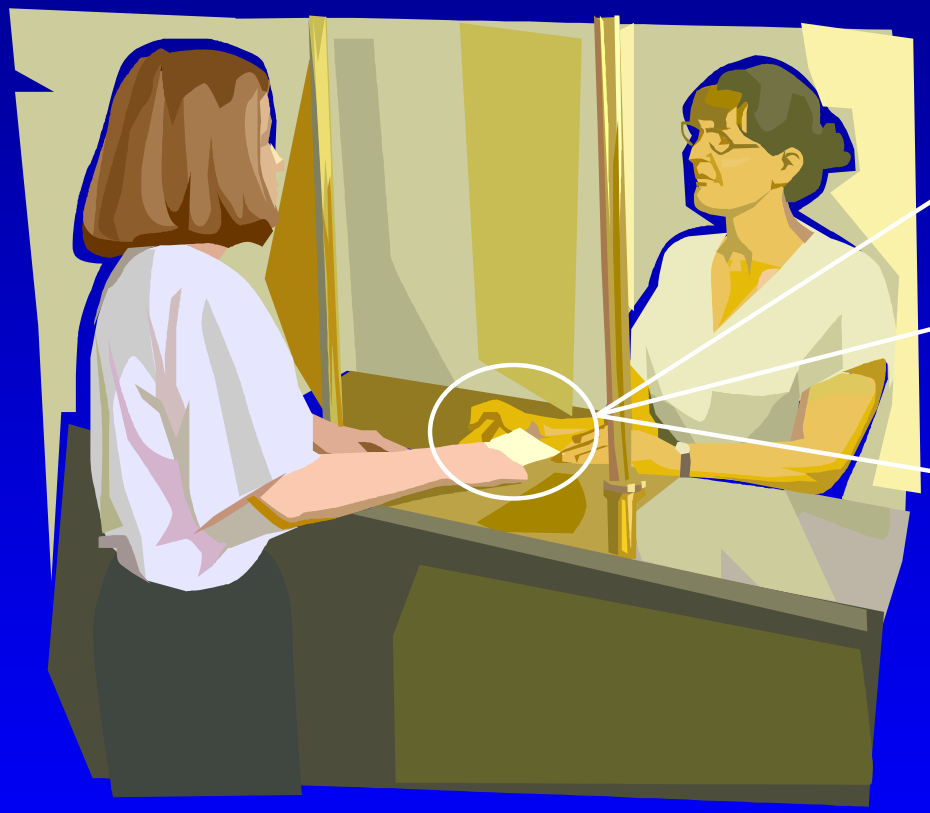
[1]http://jimwatsonsequence.cshl.edu
[2]http://www.jcvi.org/research/huref/

Predictive gene sets for response and toxicity of different therapies used in CRC.



Gil-Bazo I World J Gastroenterol 2007,13,5888-901

Personalised Medicine



DNA chip

Electronic health record

Insurance card

?

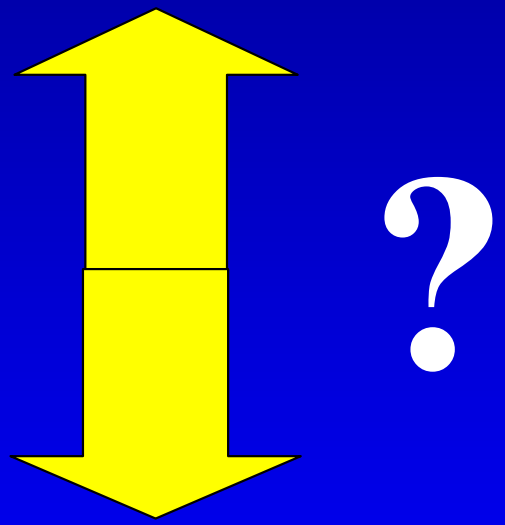
Biomarkers/Genetics: benefits for trial design

- Increase homogeneity of trial populations
- Reduce sample size required
- Clarify therapeutic benefit
- Reduce trial costs
- More rapid evaluation of new drugs

Genetically determined disease

- **Monogenic:** FAP
  sickle cell anaemia
- **Polygenic:** hypertension
  obesity
  diabetes
  schizophrenia
  cachexia?

Genotype



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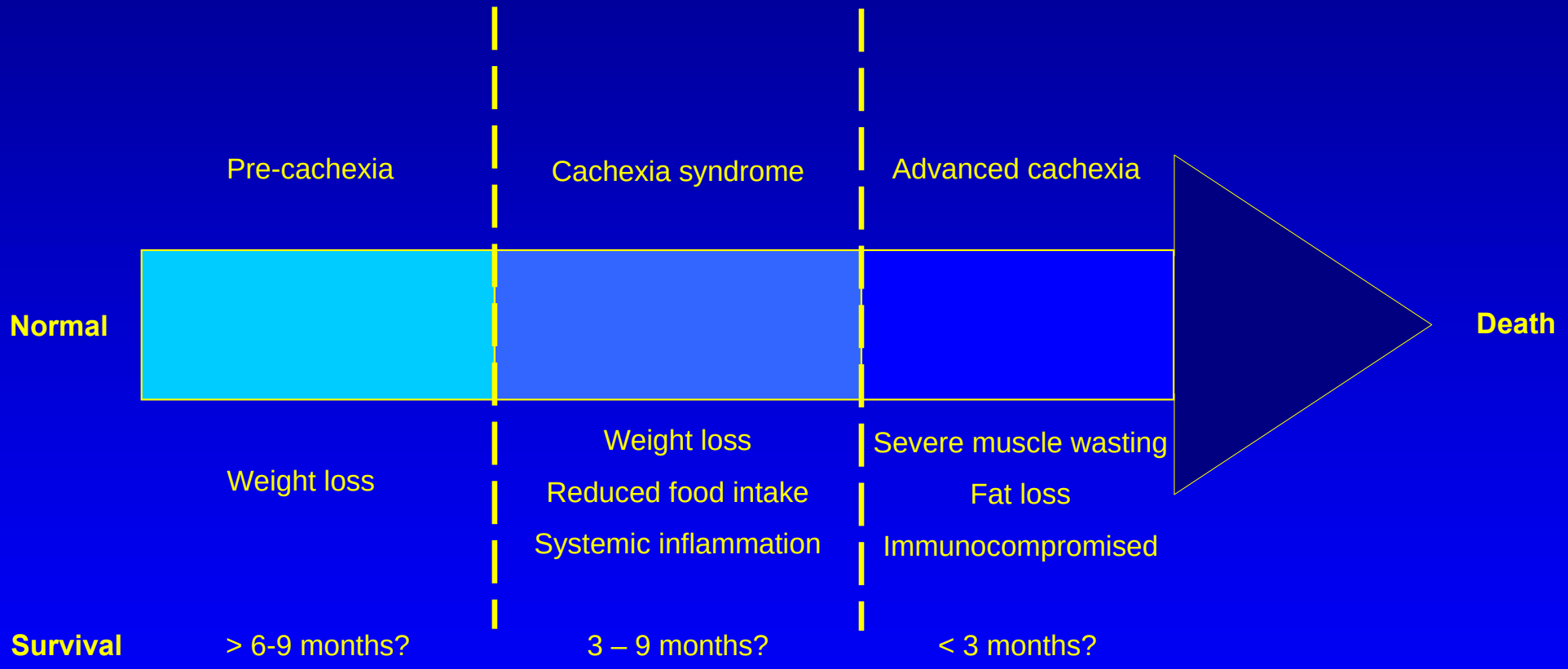
Phenotype

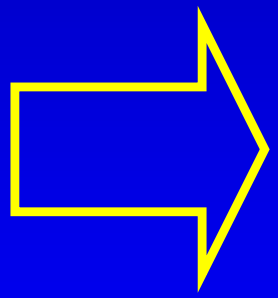
(Cachexia)

Classification of Cachexia: Cachexia represents a spectrum. Not all patients will progress down the spectrum. There are no robust biomarkers to identify those in the pre-cachectic phase who are likely to complete the journey or the rate at which they will do so.



Genetic susceptibility in cancer

Within 3 months of the end of life perhaps 1 in 6 with OG cancer are cachexia resistant



genetic polymorphisms that underline differential resistance?

Hazard ratio for risk of cachexia (weight loss >10%) stratified by IL-10 genotype (Cox's univariate regression model).

	Hazard Ratio	95% confidence interval	P value
AG *versus* AA	1.3	1.12 to 1.94	0.019
GG *versus* AA	2.3	1.18 to 4.30	0.014

Multiple regression analysis of variables associated with increased weight loss

	Hazard Ratio	95% confidence interval	P value
Dietary intake	3.9	2.32 to 5.57	<0.001
CRP conc (ln)	0.8	0.04 to 1.62	0.041
IL-10 genotype	1.9	0.46 to 3.41	0.010