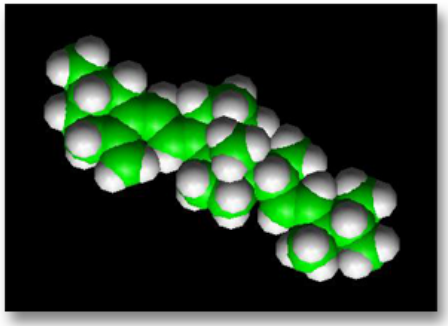


Vitamin D molecule

Chronobiology of Vitamin D:

...implications for human cancer

Dalibor V a l i k

Masaryk Memorial Cancer Institute, Brno

...vitamin D: where it came from

- in 1919/20, Sir Edward Mellanby (1884-1955),
  - *...working with growing dogs raised exclusively indoors, who developed bone disease „**rickets**“, devised a **diet** that allowed him to unequivocally establish that this disease was caused by a **deficiency of a trace component** present in the diet.*

…in 1921 he wrote:

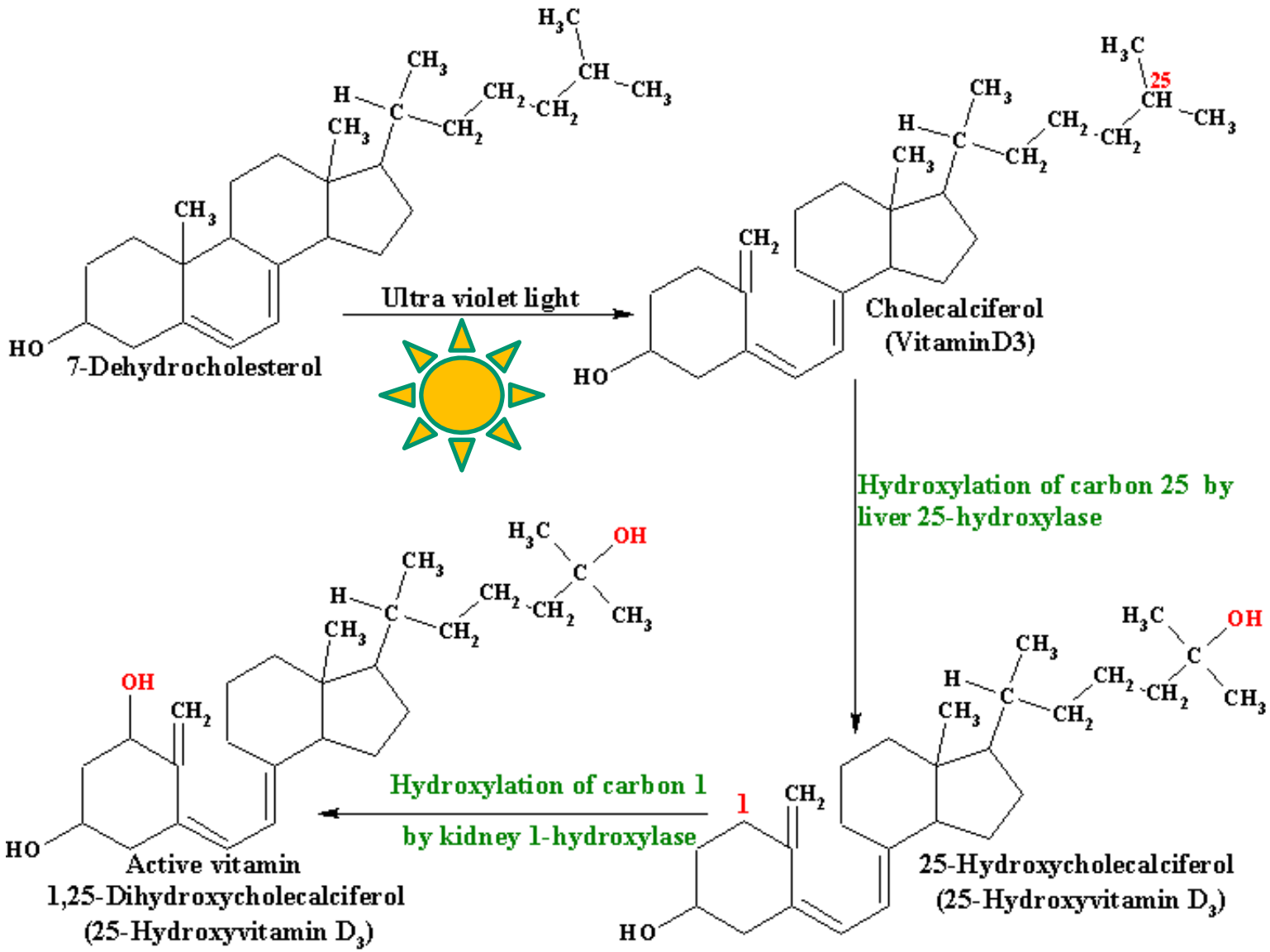
- *„…The action of fats in rickets is due to a vitamin or accessory food factor, which they contain, probably identical with the fat-soluble vitamin.„*

- …later on, he established that **cod liver oil** was an excellent antirachitic agent.

» ……x-ray of florid rickets >

Biosynthesis

...in the presence of sunlight



Seasonality in oncology: lessons from history

- **1941** Apperly FL
  - - cancer mortality connected to sunlight
- **1981** Abe et al
  - – **vitamin D role** in diferentiation of CML cells and a growth inhibition of malignant melanoma cells
- **2005-2007 animal models**
  - – prodifferentiation effect of vitamin D on hyperproliferative processes
- **2000-2007** epidemiological studies
  - – publishing an inverse relationship between vitamin D intake and colorectal cancer
- **2010** Jenab et al
  - – metaanalysis of 35 prospective studies- inverse relationship between 25-OHD plasma levels and CRC risk

...blood levels of 25-OHD and CRC

- **Metaanalysis by Gorham, ED, et al 2007**
- 25-OHD levels > 82 nmol/l
  - ...resulted in 50 % lower incidence of CRC than levels of vitamin D < 30 nmol/l.

- **Dana-Farber Cancer Ng,K et al 2008 n=304**
  - ...patients with higher „prediagnostic“ levels of 25-OHD resulted in about 50 % reduction of death

...OUR EPIDEMIOLOGICAL RESEARCH

...a research question:

is there seasonality pattern in incidence of cancer ?

... = yes, it is.

- C18-C20
- C25
- C50
- C56
- C61
- C64
- C43

Seasonality detected in incidence profile of selected tumours

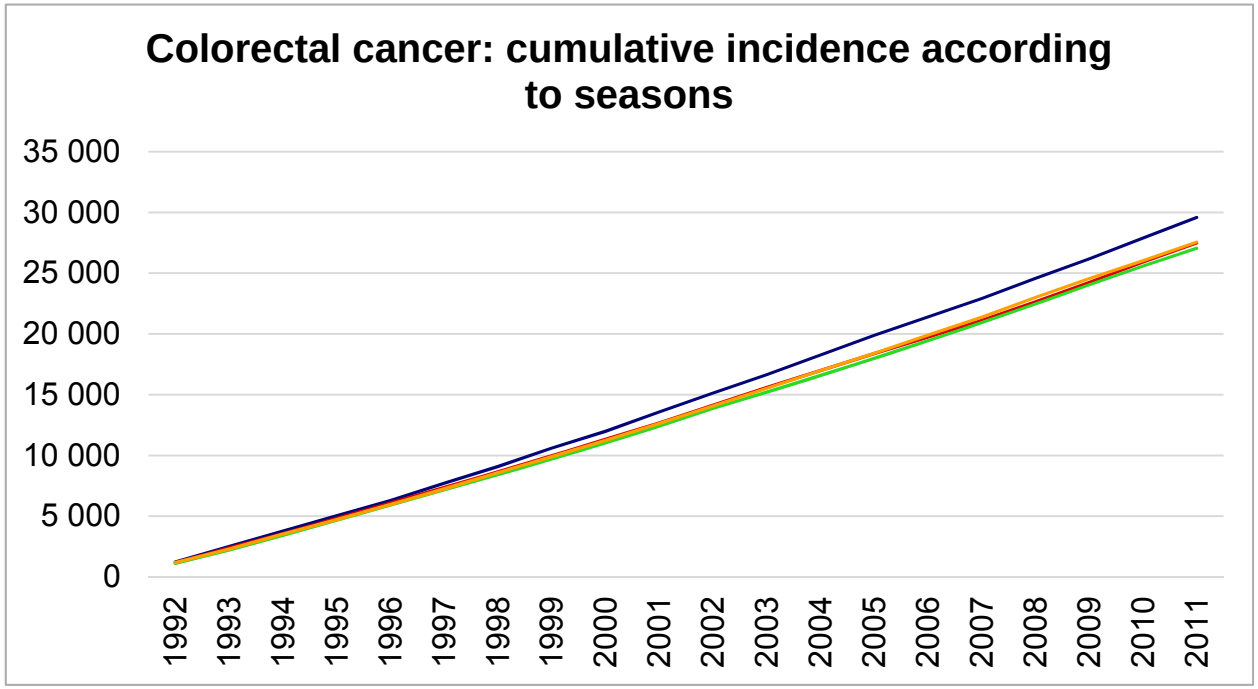
Diagnosis	Observed incidence pattern
Colorectal cancer (C18-C20)	Significantly increased incidence in spring and decreased in autumn
Pancreatic cancer (C25)	No observed seasonal pattern
Breast cancer (C50)	Significantly increased incidence in spring and decreased in October
Ovarian cancer (C56)	Increased incidence in March
Prostate cancer (C61)	Significantly increased incidence in spring and decreased in September
Kidney cancer (C64)	Increased incidence in spring and decreased in autumn
Malignant melanoma (C43)	Highly significantly increased incidence in spring and summer, decreased in autumn and winter

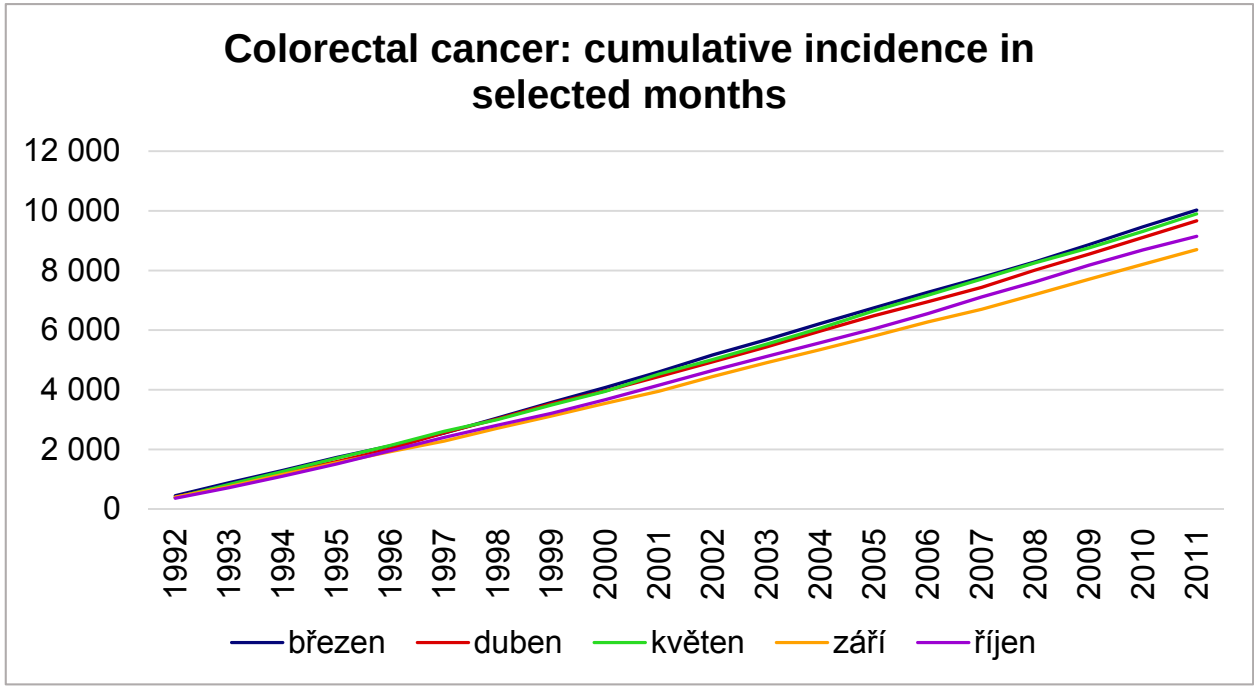
methodological approach to analyze seasonal patterns

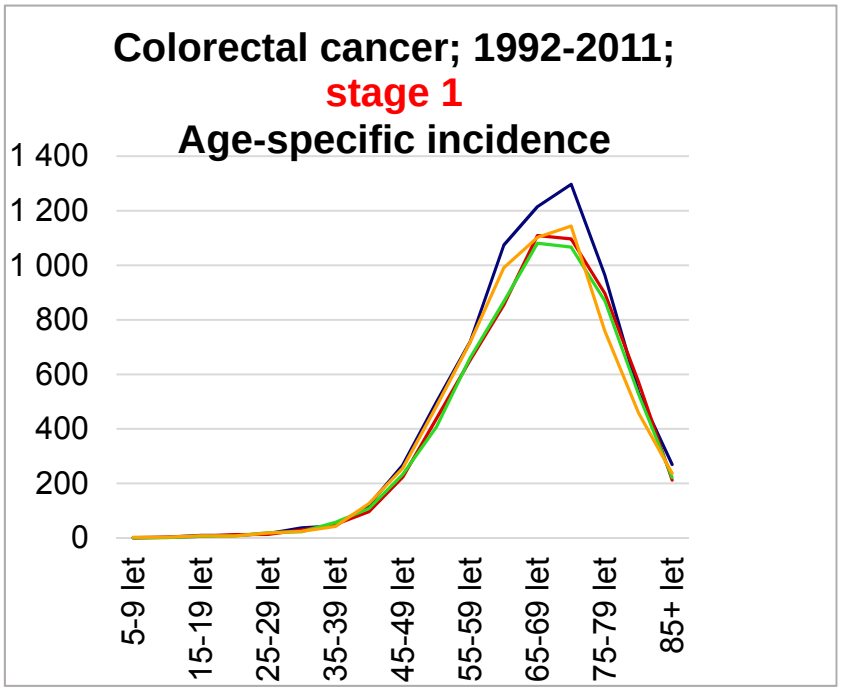
- 1. Cumulative incidence mass was calculated over 20 years
    - **(i.e. all Januaries through all Decembers across 20 yrs)**
  - => this integrated approach is robust in analysis of seasonal differences, since annual fluctuations partially mask seasonal patterns

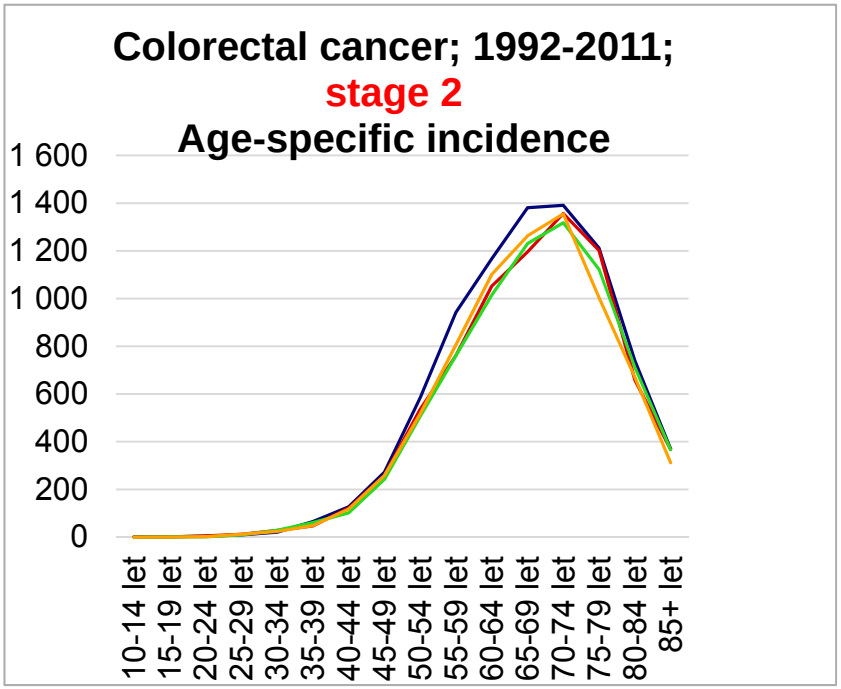
- 2. Age-specific and stage-specific profiles of incidence were obtained
  - „spring“ was defined as March+April+May, i.e. not astronomical spring
    - (reporting is obviously by months)

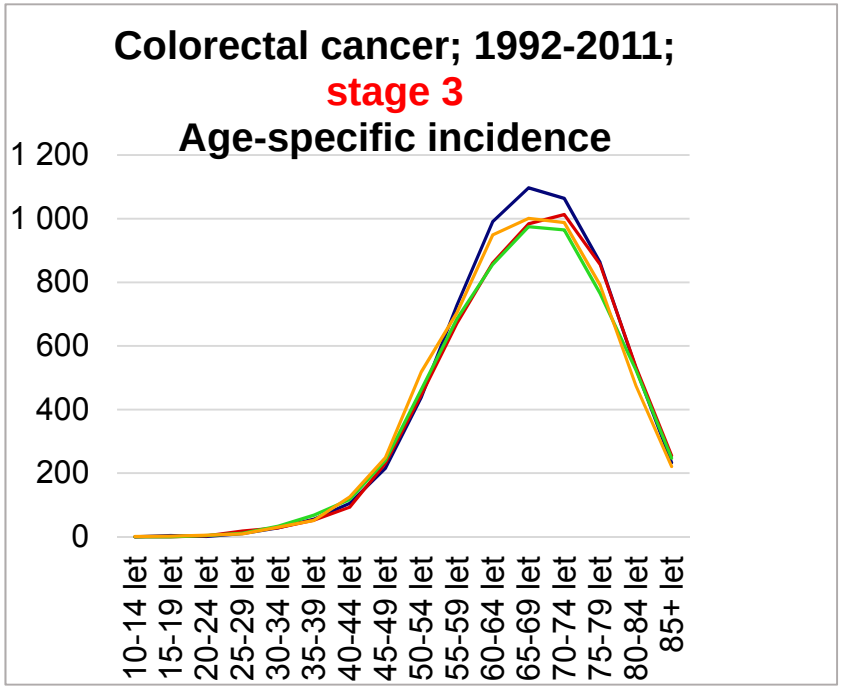
Cumulative incidence profile: **colorectal cancer**

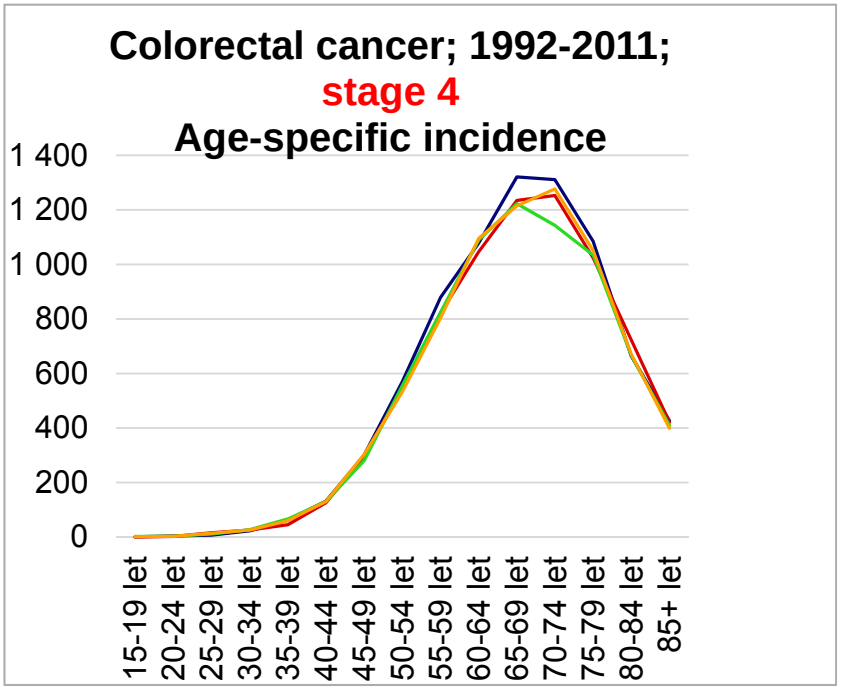










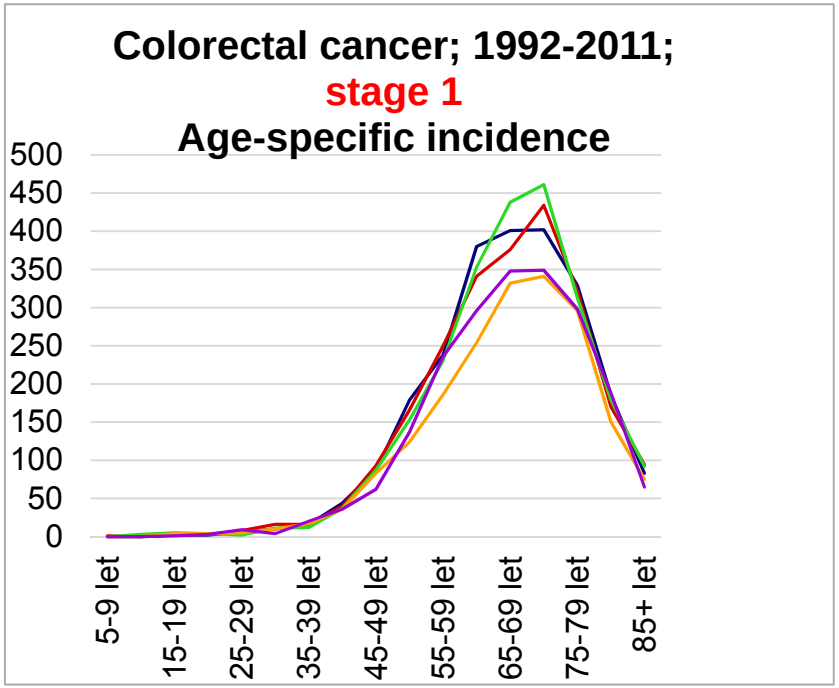


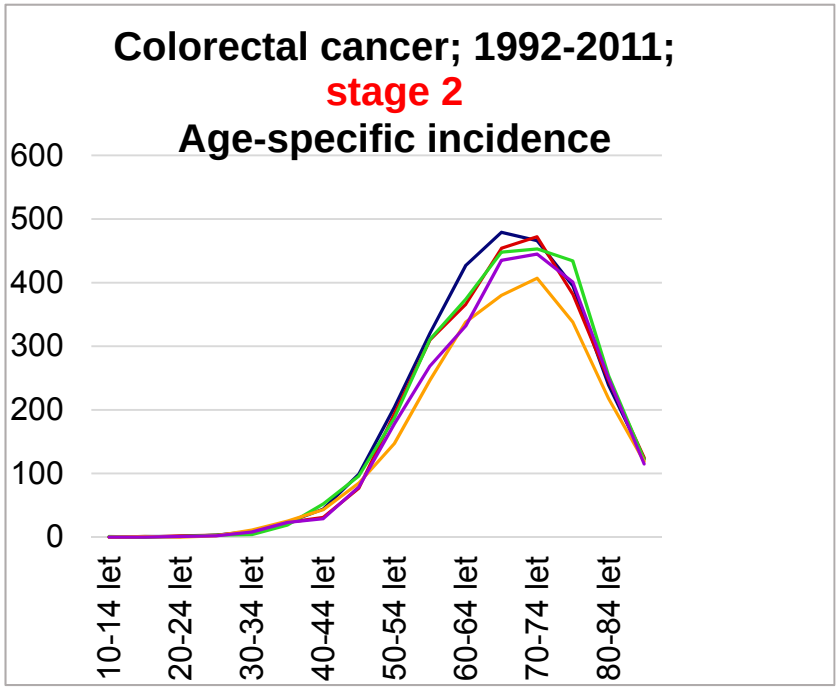
Spring

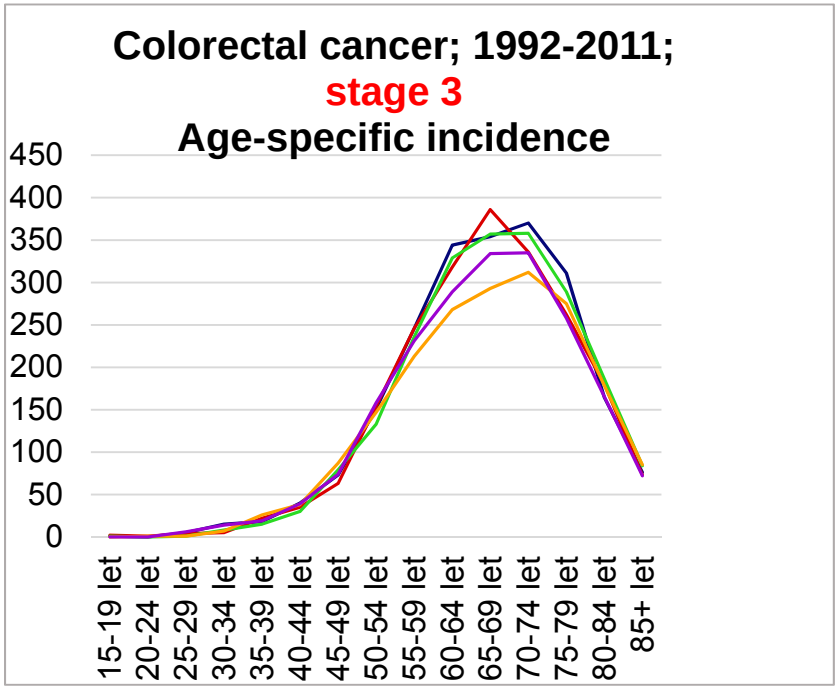
Summer

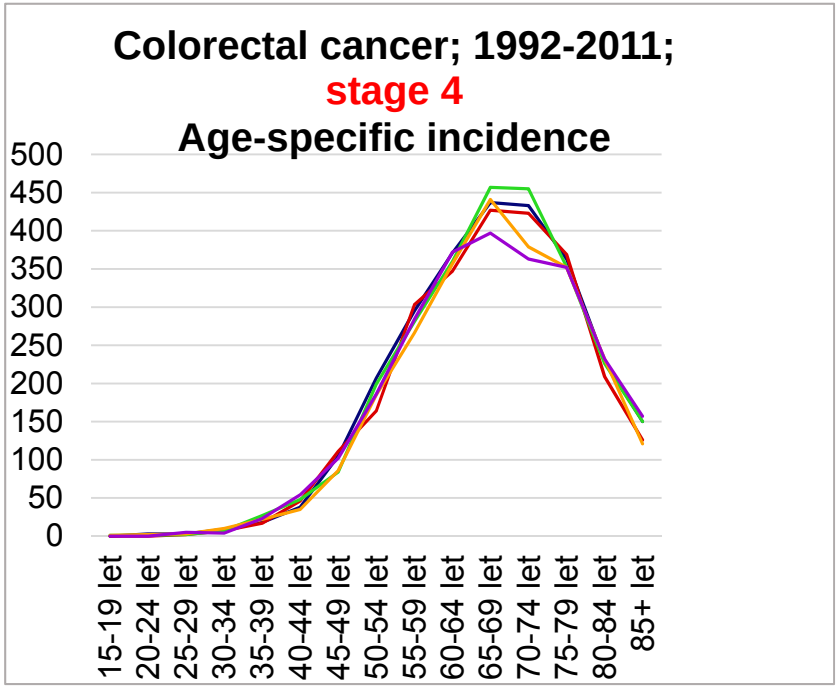
Autumn

Winter



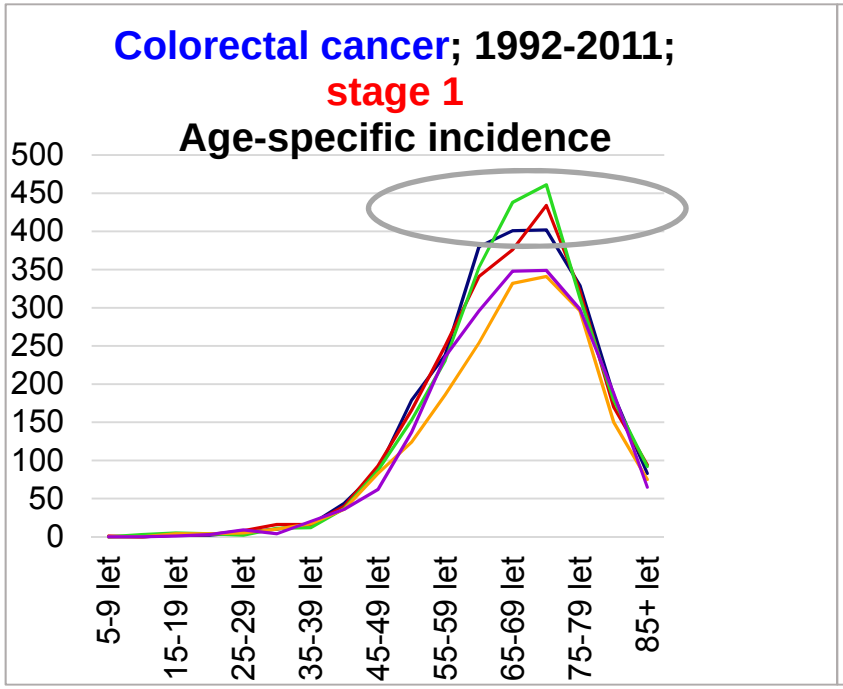


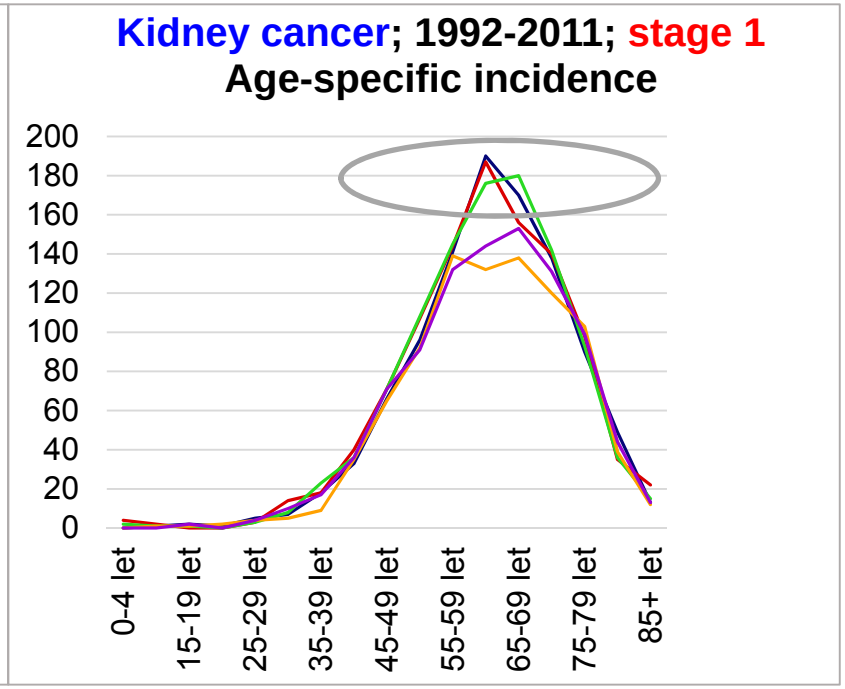




March
April
May
Septemb
October

…we observed most significant differences ($p < 0.001$) for CRC and RCC





March

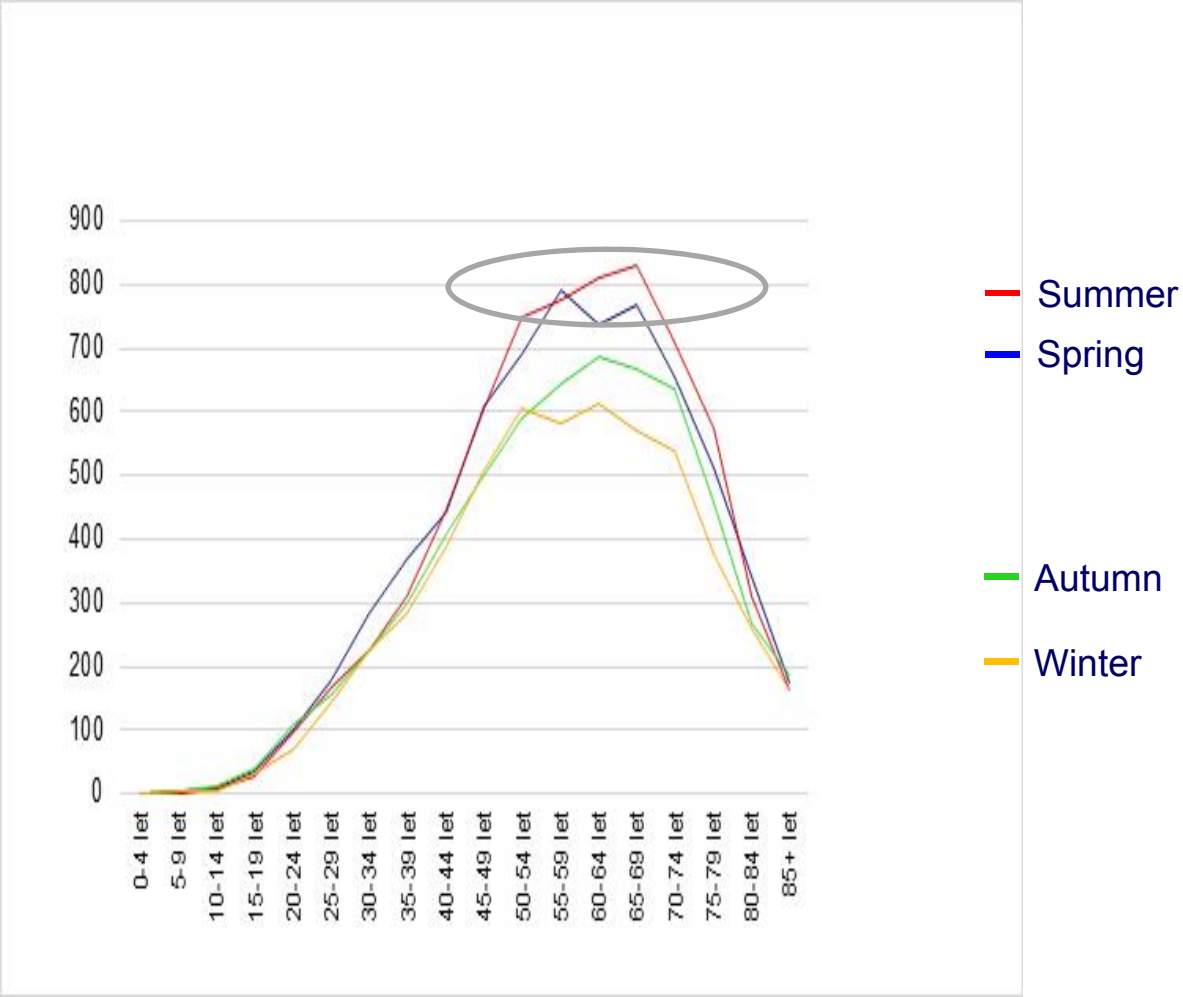
April

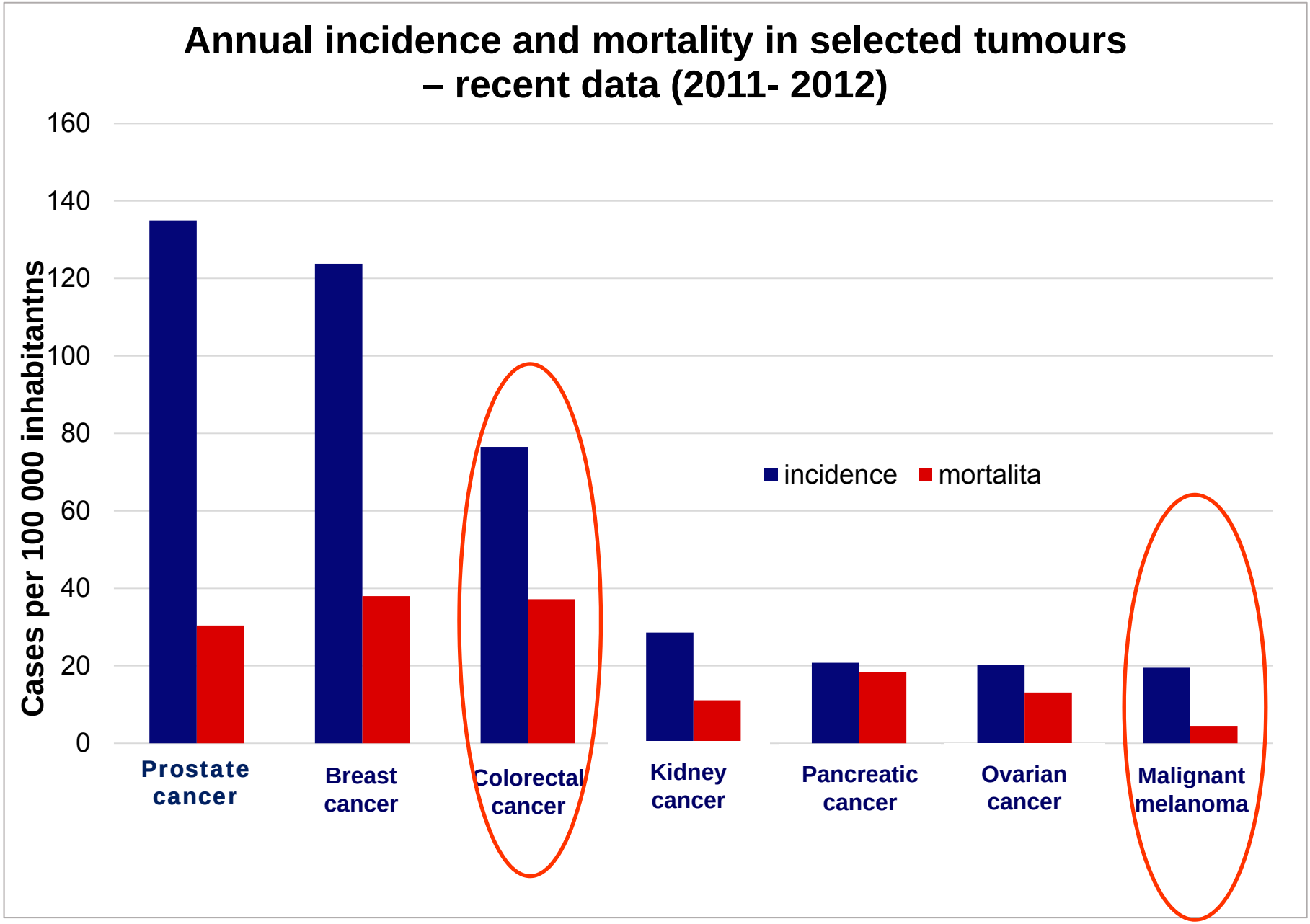
May

Septem.

October

Malignant melanoma – …peaking in summer (p < 0.001)




Annual incidence and mortality in selected tumours
– recent data (2011- 2012)
Cases per 100 000 inhabitantns
160
140
120
100
80
60
40
20
0
incidence
mortalita
Prostate cancer
Breast cancer
Colorectal cancer
Kidney cancer
Pancreatic cancer
Ovarian cancer
Malignant melanoma

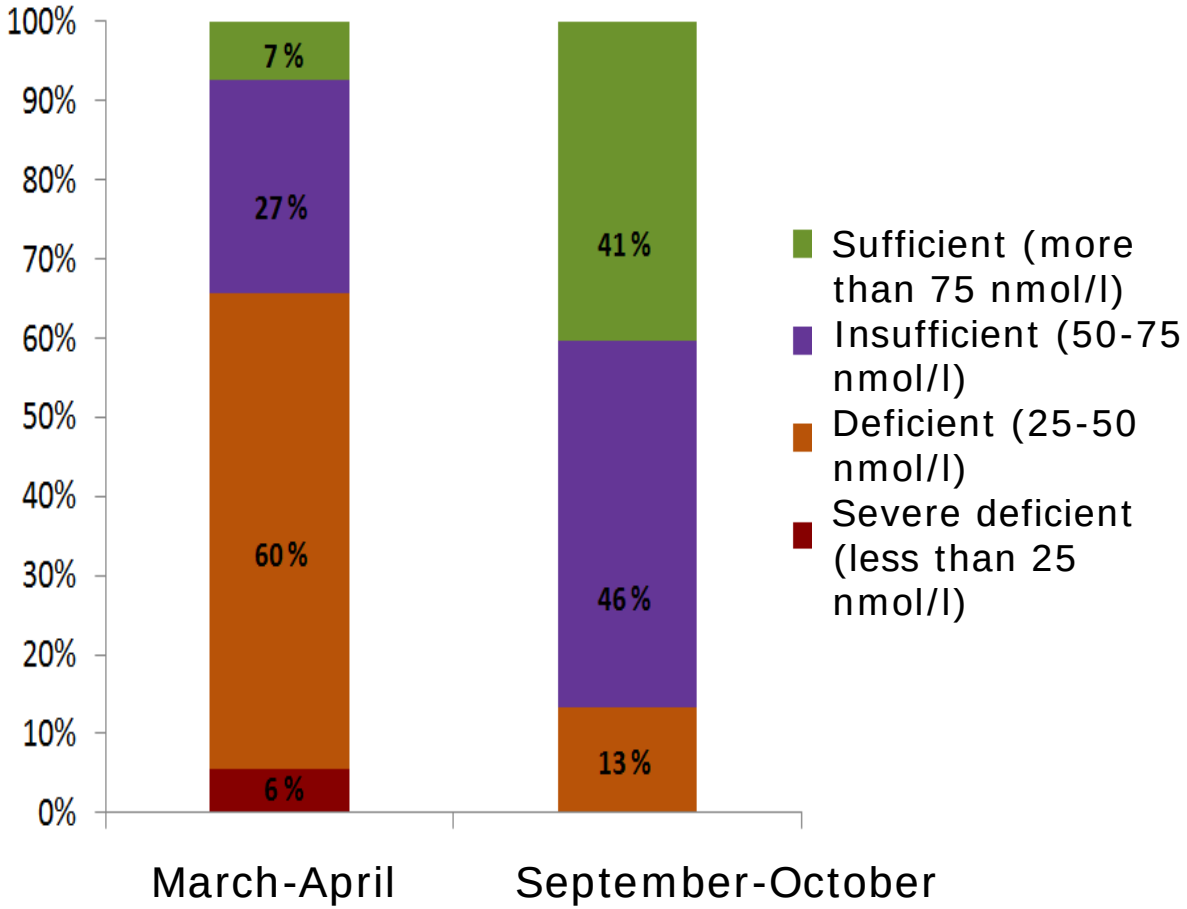
...OUR EPIDEMIOLOGICAL AND LABORATORY RESEARCH

...circannual pattern of blood 25-OHD levels, does it exist ?

- spring cohort,
437 observations from March+April:
-mean 42,7 nmol/l (95% confidence interval 21,9–90,1 nmol/l)

- autumn cohort,
508 observations from Sept.+Oct.:
-mean 70,8 nmol/l (95% CI 37,0-137,5 nmol/l)

-ARCHITECT i2000sr (Abbott)

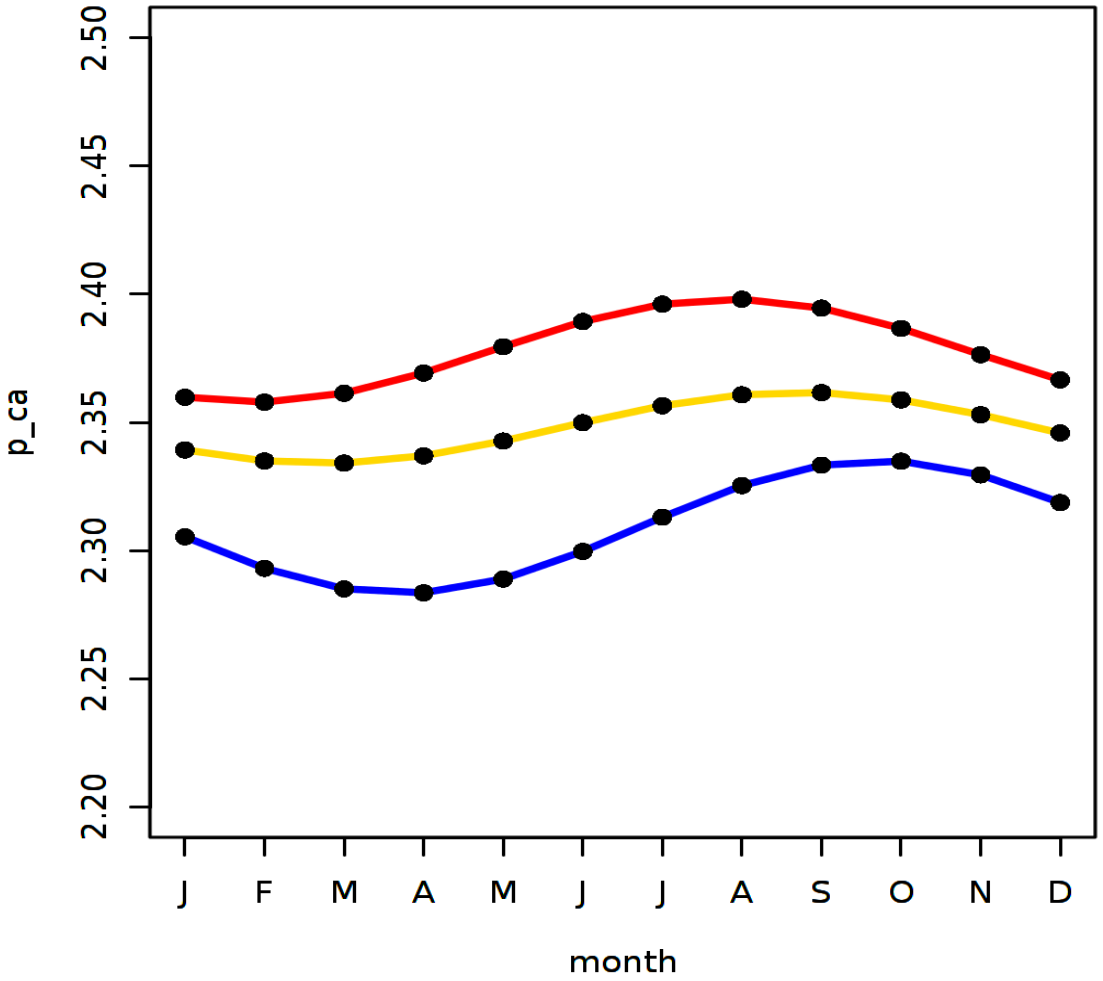


=> interpretation: yes, levels vary with cca 90 % amplitude and cca 6 months frequency interval

...then, how to validate it ?

validation question: what is the variability of vitD-regulated biomolecule ?

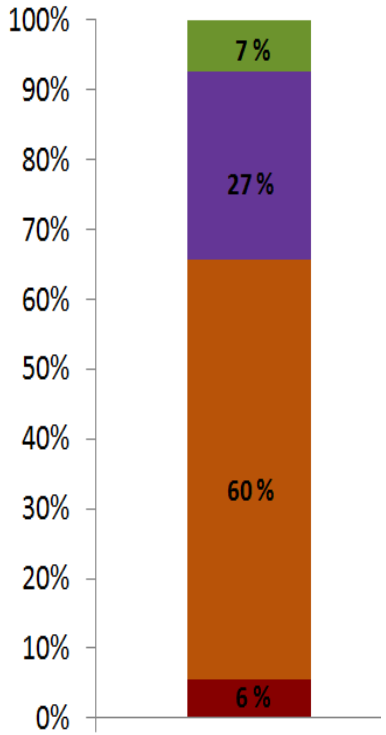
=>...annual p_Ca variability (median):



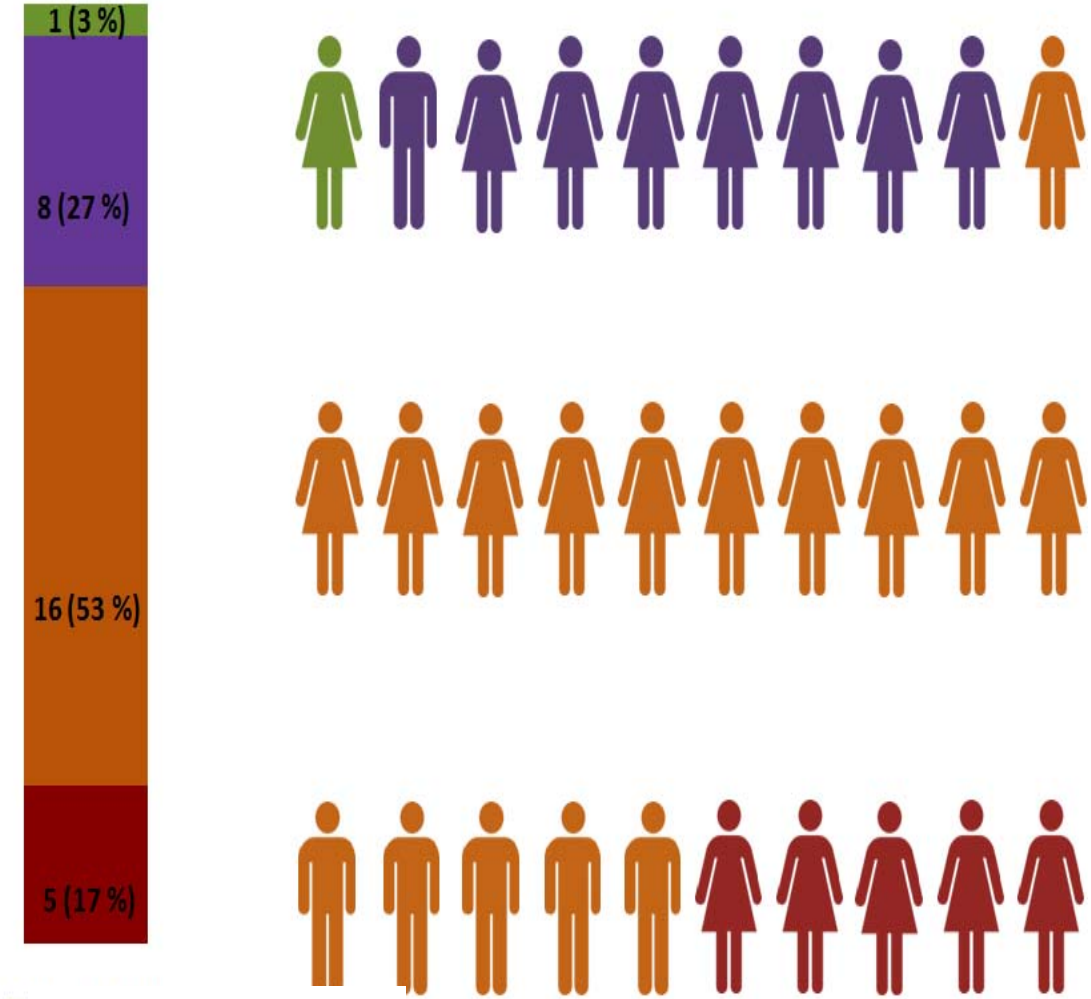
Red: C50, n=1040
gold: healthy, n=31982
blue: C18,19,20, n=763

…and how to validate it further ? staff levels of 25-OHD in spring

Reference cohort 437 obs.



March-April

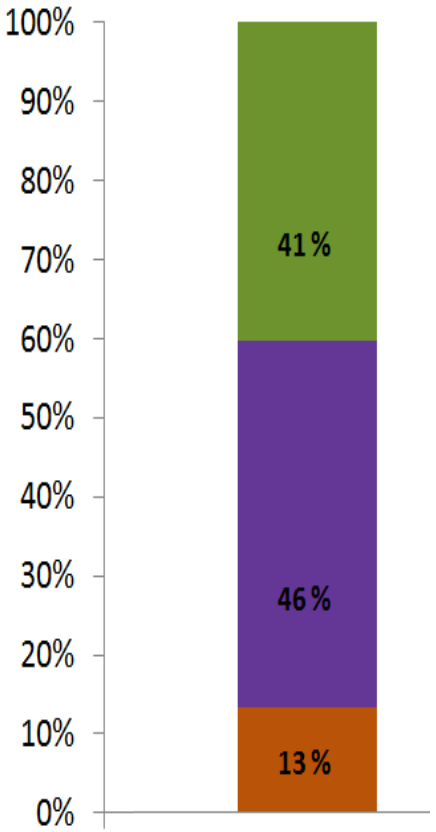


Sufficient
Insufficient
Difficient
Severe defficient

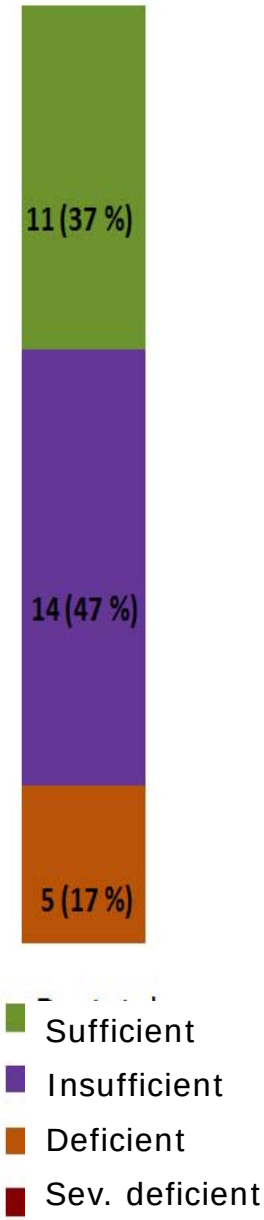
March-April

...And in autumn

Reference cohort 503 obs.



September-October



September-October

...interim summary I

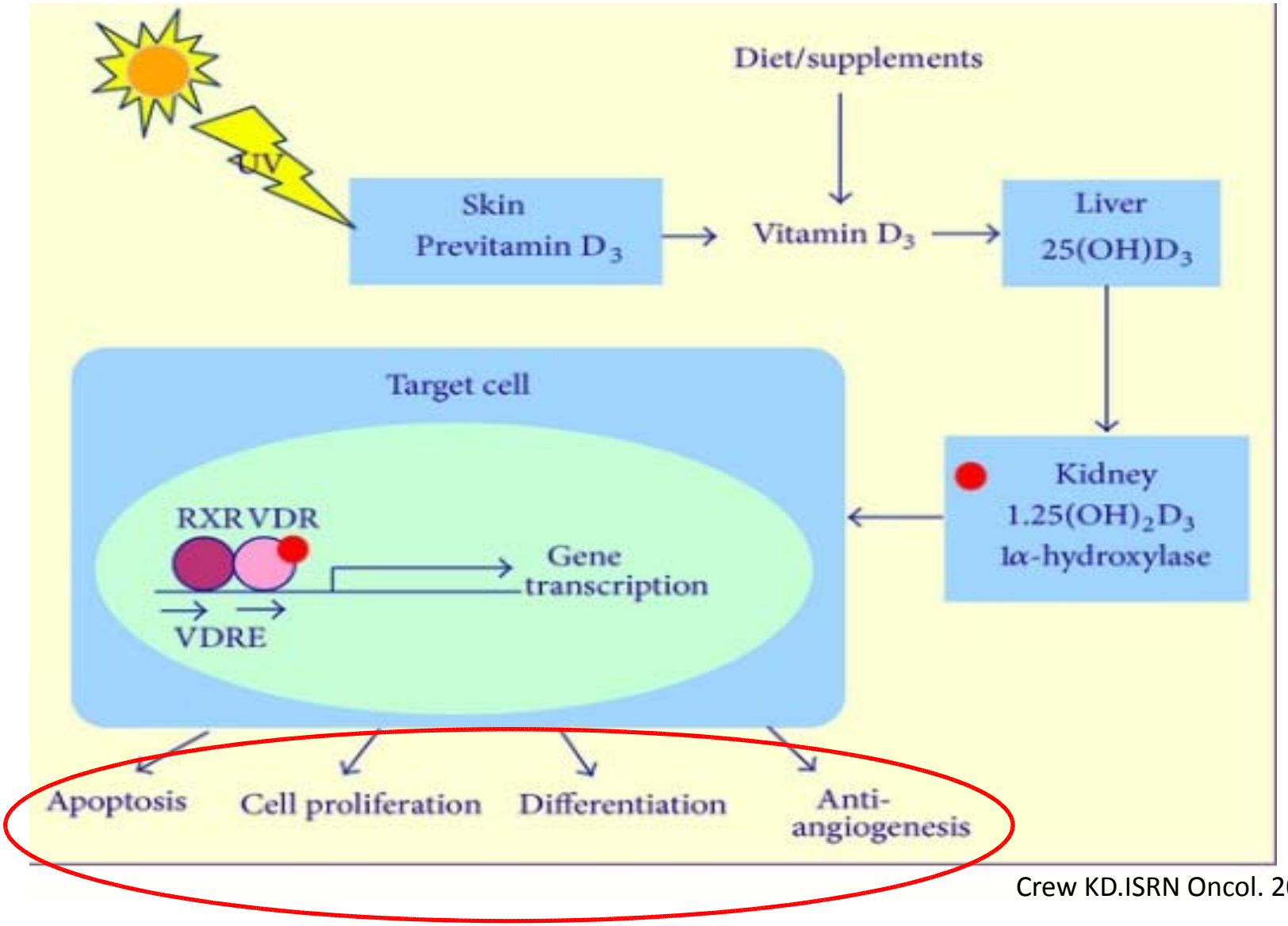
- ...on the basis of epidemiological and laboratory data one can indeed conclude that **circannual variations of vitamin D blood levels** along with vitamin D-regulated bioactive molecules were
  - i) **robustly established and validated** thereof and
  - ii) **consistently projected in the seasonal cancer incidence curves** being mostly apparent for
    - **iii) CRC and RCC**
    - **whilst inversely apparent for melanoma**

...OUR EPIDEMIOLOGICAL, LABORATORY AND CLINICAL RESEARCH

...how vitamin D exerts its biological effects

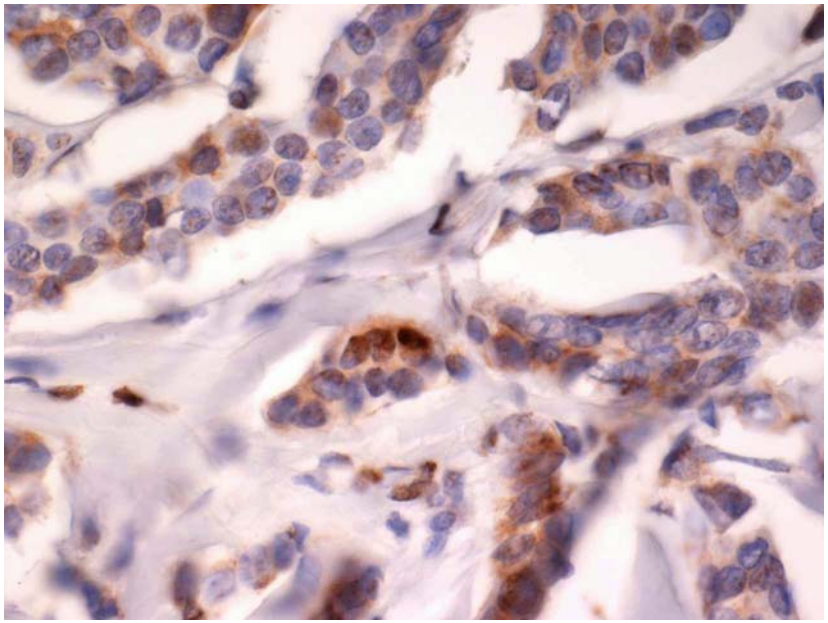
- ...genomic and nongenomic effects
  - nongenomic = through membrane bound receptor-type molecules, yet poorly characterized
    - » ...rapid insulin release
    - » ...rapid calcium absorption in the interstine
  - genomic = nuclear receptor-mediated response:
    - » ...through VDR, a „new“ member of steroid hormone receptor superfamily

…brief vitamin D physiology overview

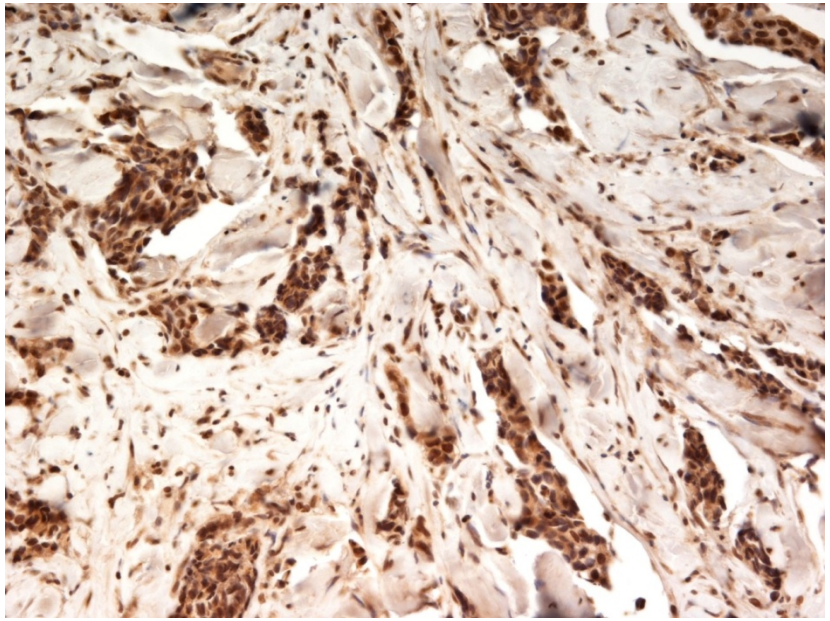


Crew KD.ISRN Oncol. 2013

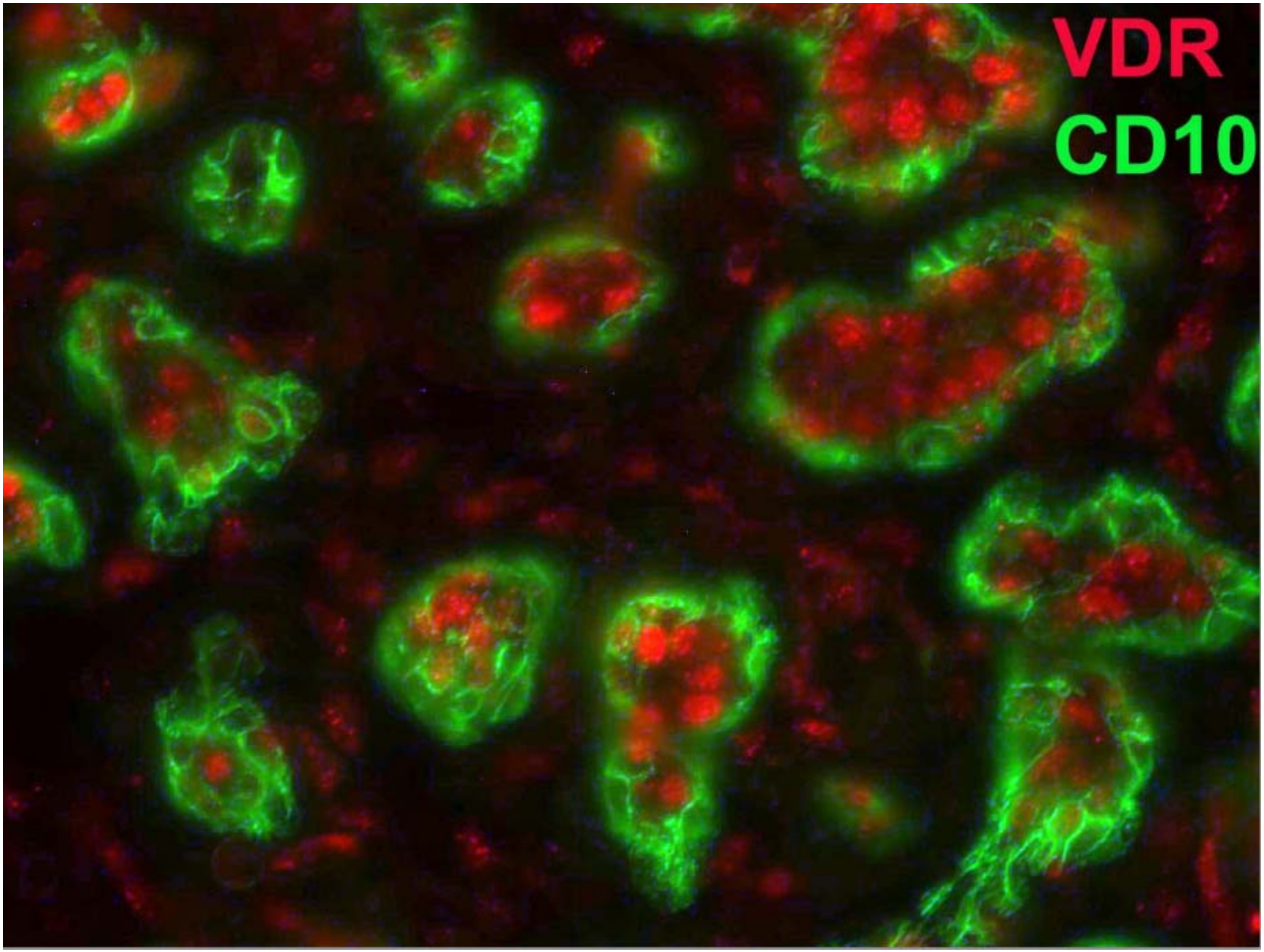
VDR expression: breast cancer



breast cancer, 400x, heterogenous epithelial and stromal staining



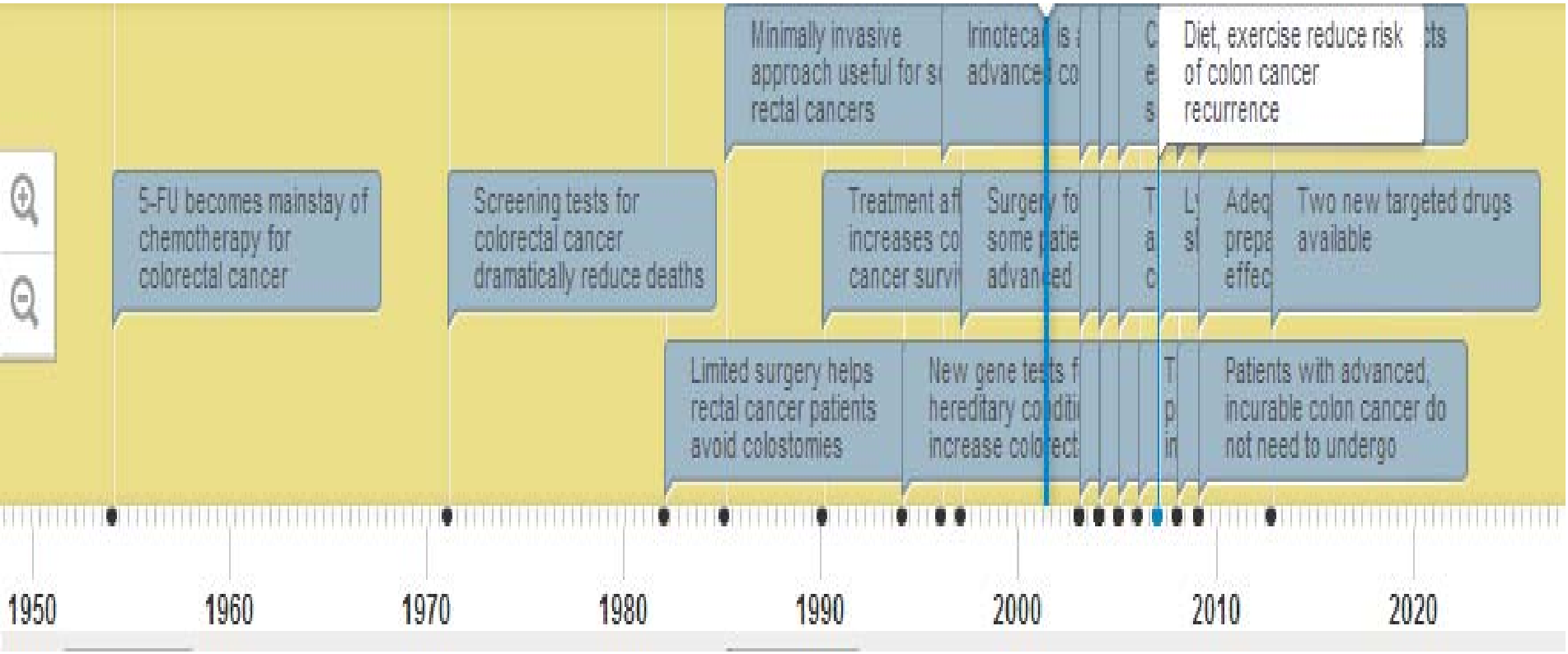
breast cancer, 100x, strong homogenous staining of epithelia and stroma, both are ER positive



VDR in normal breast, more staining in luminal cells (different antibody used)
Most of ER+ positive cancers are VDR+ *J Clin Invest. 2014;124(2):859–870.*

mCRC timeline

Vitamin D and prospective trials



...so what can we learn from ?

*...2007: Patients who follow a **low-fat diet and exercise** regularly are found to have a **lower risk of colon cancer recurrence** after surgery for early-stage disease demonstrating that lifestyle factors can have a significant impact on cancer recurrence. The results provide patients with new tools for reducing the risk that their cancer will return...*

...a questions is then:

*.....Individuals with **higher blood 25-OHD** levels have a **lower risk of developing colorectal cancer**, but the **influence of 25-OHD** on mortality **after CRC diagnosis** is unknown.*

Time-course patterns of blood 25-hydroxycholecalciferol are significant predictors of survival outcome in metastatic colorectal cancer: clinical practice-based, proof-of-principle study,

Valik et al, Neoplasma, accepted

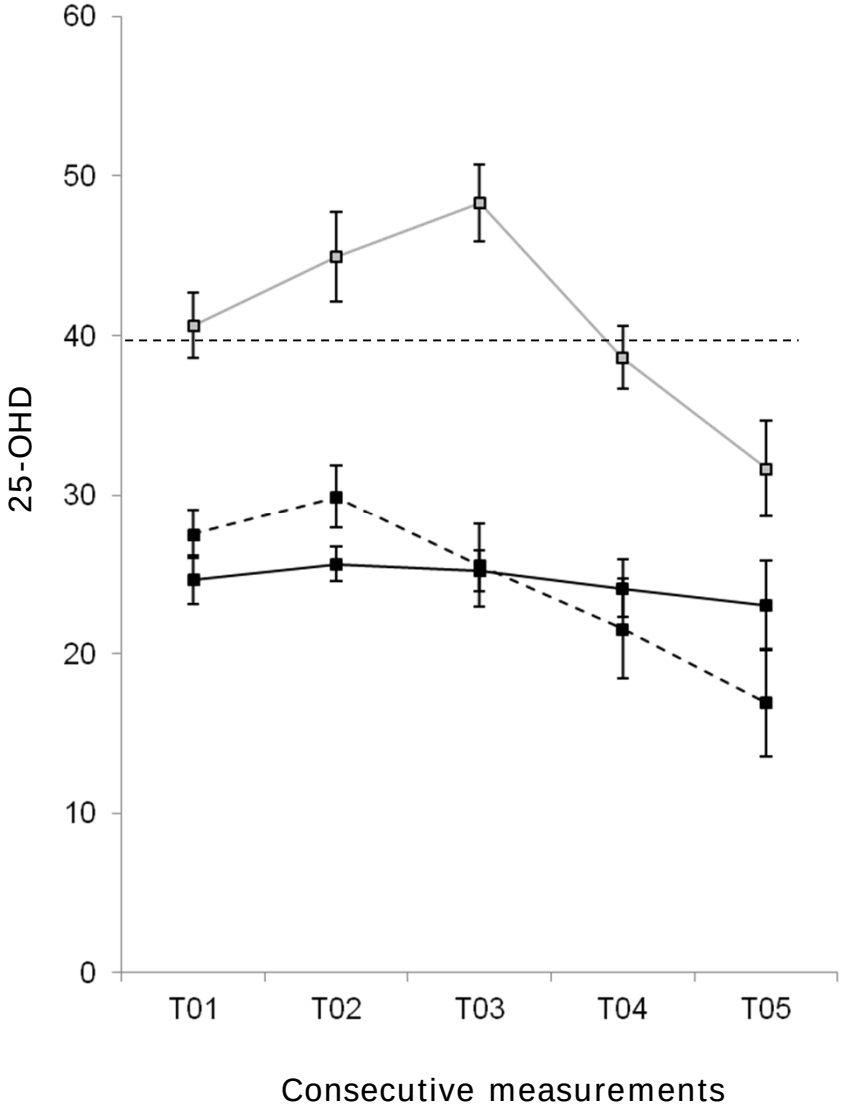
- We studied blood levels of 25-hydroxycholecalciferol in relation to other clinical and laboratory variables in metastatic colorectal cancer patients to ascertain whether their variations may be a prognostic or predictive parameters of survival outcomes

Patient characteristic

- We included 84 patients treated with first-line oxaliplatin-based chemotherapy with or without bevacizumab.

- The patients were enrolled on the intent-to-treat basis considering their performance status, comorbidities and laboratory parameters to be medically apt for intensive chemotherapy.
    - The study was made possible due to having a **research BBMRI-affiliated biobank** at MMCI storing besides tissues also periodical aliquots of patient sera from CEA determinations

Time-course typology patterns of 25-OHD blood levels during therapy of metastatic CRC



Arithmetic mean

Standard error

Number of samples in given time	T01	T02	T03	T04	T05
At least 1 25-OHD value 40 or above	40	40	32	24	8
All 25-OHD < 40 - fluctuating	14	14	12	9	6
All 25-OHD < 40 - stable	30	30	15	11	7

Potential predictors of OS in Cox proportional hazard regression model

Endpoint: overall survival		Univariate estimates		Multivariate-adjusted estimates	
		HR (95% CI)	p	HR (95% CI)	p
Any surgical procedure		0.239 (0.098; 0.583)	0.002	0.240 (0.097; 0.594)	0.002
Radical resection of metastases		0.101 (0.014; 0.747)	0.025		
25-OHD	25-OHD (max) < 40	2.238 (1.029; 4.868)	0.042		
	25-OHD (max) < 50	3.178 (1.107; 9.068)	0.032		
	All 25-OHD in time series < 50	3.344 (1.168; 9.576)	0.024		
	All 25-OHD in time series < 40	2.466 (1.133; 5.371)	0.023		
Typology of 25-OHD time series (binary code)	All values <40 – stable, without any elevation	2.710 (1.333; 5.510)	0.006	2.220 (1.074; 4.592)	0.031
CEA	CEA (first) > 11	4.610 (2.115; 10.051)	<0.001	4.862 (2.196; 10.762)	<0.001

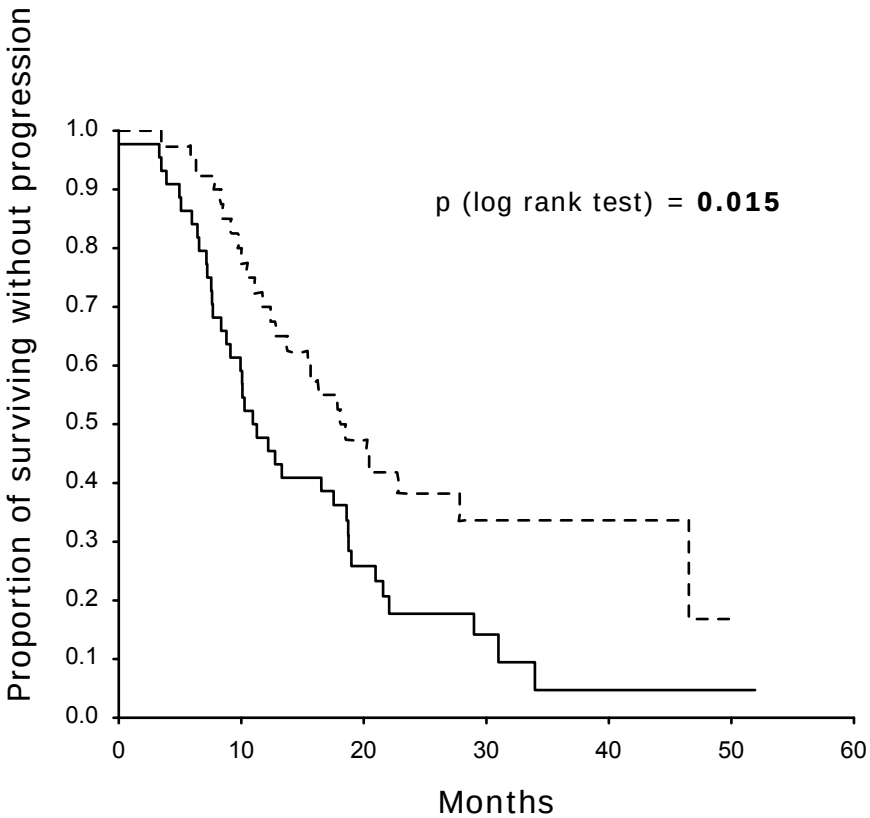
Survival of patients according to time-course typology patterns of 25-OHD blood levels (nmol/l)

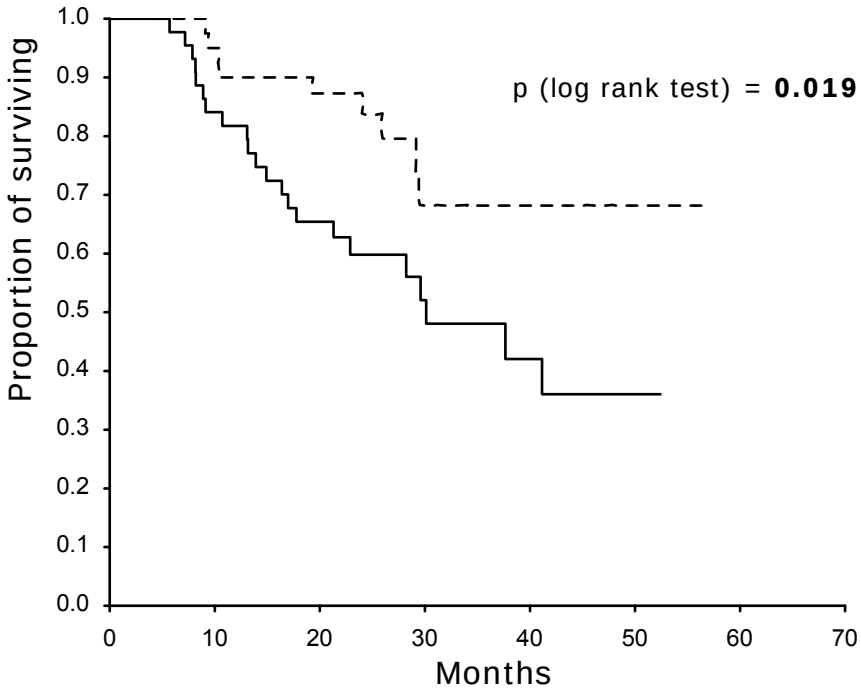
--- **NORMAL (N)**

—— **LOW (L)**

(N):At least one 25-OHD value 40 or above (N=40)

(L): All 25-OHD < 40 (N=44)





No at risk	Time 0	12	24	36	48
N	40	28	11	2	1
L	44	21	5	1	1

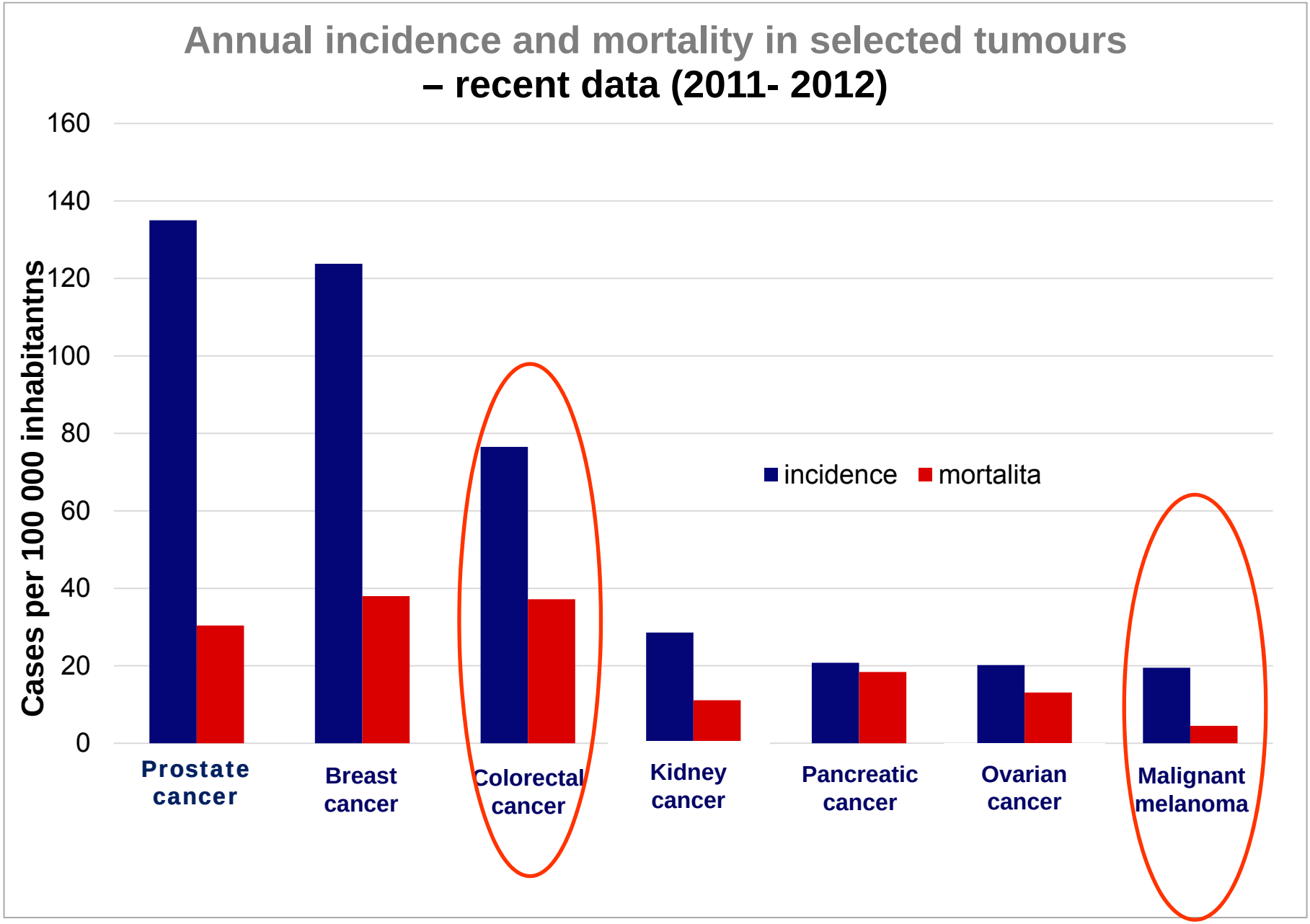
No at risk	Time 0	12	24	36	48
N	40	36	24	5	3
L	44	35	18	8	2

...interim summary II

- **Vitamin D is a pleiotropic bioactive molecule that exerts influence on the course of active disease. Its plasmatic levels assessed as trend patterns, but not single samplings, possess robust predictive power towards mCRC outcome.**

- **Although it is tempting to speculate that pharmacological administration of cholecalciferol may have favorable effect on the disease clinical course, this assumption needs to be proven under clinical trial conditions.**

- **From the point of oral administration, clinical fear of overdosing is probably very unsubstantiated, cholecalciferol prepraration are probably safe to up to 10000 IU/day.**


Annual incidence and mortality in selected tumours
– recent data (2011- 2012)
Cases per 100 000 inhabitantns
160
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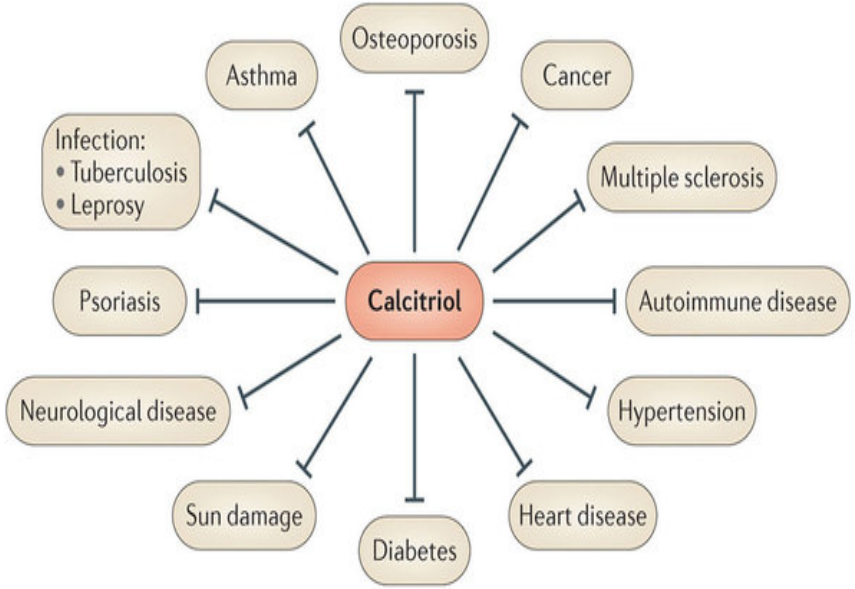
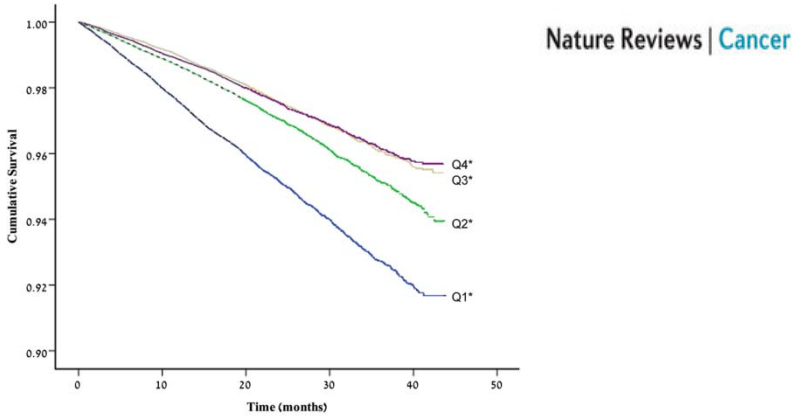
80
60
40
20
0
incidence
mortalita
Prostate cancer
Breast cancer
Colorectal cancer
Kidney cancer
Pancreatic cancer
Ovarian cancer
Malignant melanoma

…vast number of diseases and **all-cause mortality** linked to vitamin D deficiency

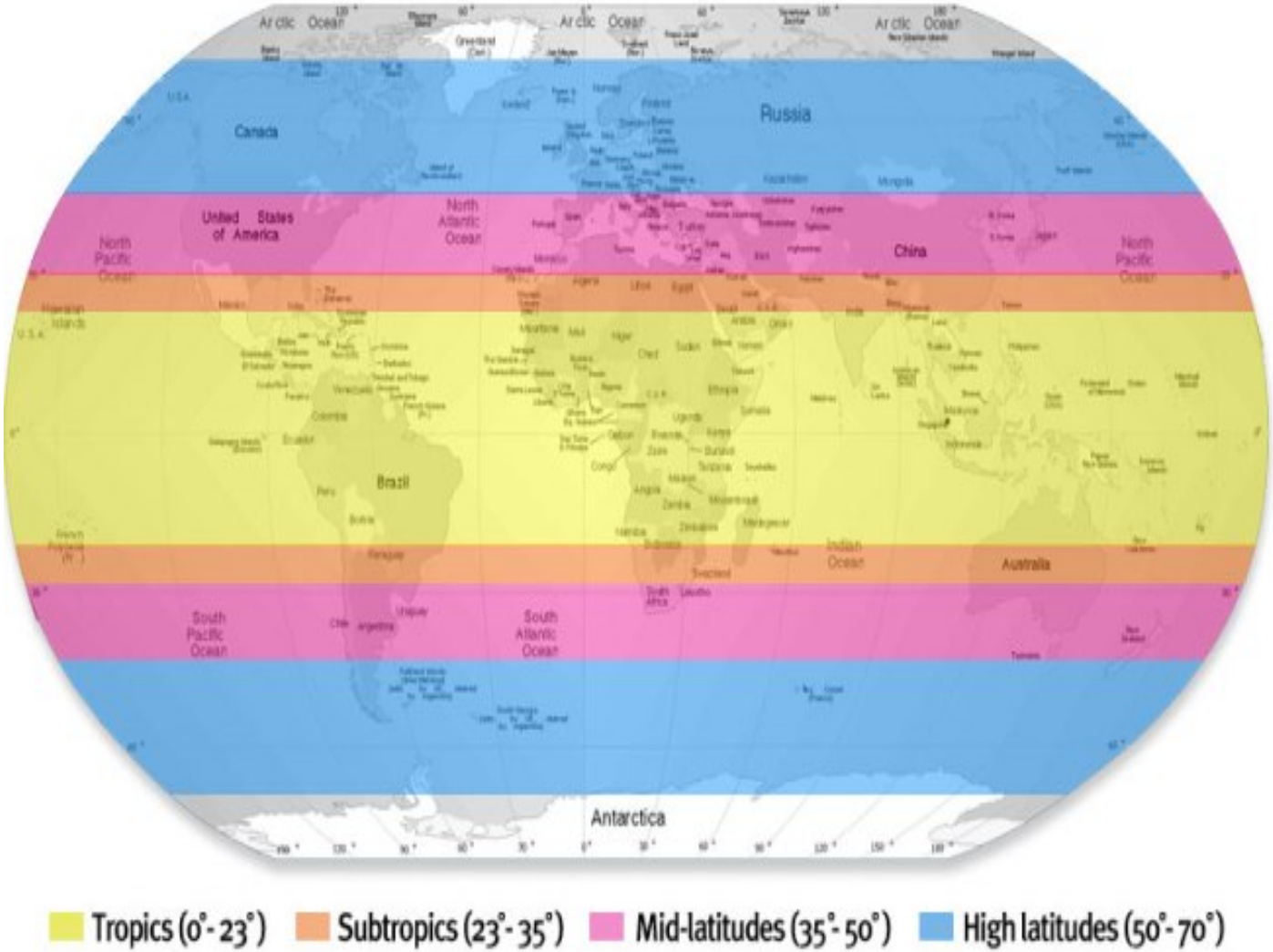
- **respiratory infections**
- **muscular weakness**
- **psoriasis**
- **diabetes** – studies from Finland: 10 366 children got 2000 IU of D3 during first day after birth, 31 years followup, risk of diabetes decreased by 80% !!
- **asthma** – studies from Japan: 1200 IU / day = less asthmatic attacks
- **parodontic diseases**
- **cardiovascular disease** – deficiency linked to high risk for hypertension
- **schizophrenia and depression**
- **cancer** – vitamin D decreases incidence and improves prognosis





*Quartiles of serum 25(OH)D
Q1 = ≤33.8 nmol/L
Q2 = >33.8 to 49.4 nmol/L
Q3 = >49.4 to 65.2 nmol/L
Q4 = >65.2 nmol/L

J Clin Endocrinol Metab, 2012, 97(8):2792–2798



- **Africa – blacks, i.e. low synthesis but outside, India – undressed outside, Arabs – dressed, Florida – in airconditioned houses and cars, Czech Republic – summer vacations in Greece and Croatia…??**

...but how to cover requirements for vitamin D

How many minutes of mid-day summer sun needed for adequate vitamin D?

			12%	26%	46%	72%
			percent skin exposed			
San Diego	Youth	Lying down	42 min	19 min	11 min	7 min
Seattle	Youth	Standing	168 min	76 min	44 min	28 min
Seattle	Senior	Standing	504 min	228 min	132 min	84 min

Adequate = **40** nanograms/ml Does not include Obese, Dark Skin

Details: http://www.**is.gd/timeinsun**

...overall summary

- **Vitamin D is a significant biological modulator of incidence of CRC and other human cancers as well**
  - » and its blood levels may be an informative prognostic factor in the course of active disease

- **Current recommendation on sun exposure may be perhaps too restrictive**
  - » mostly being postulated on skin cancer incidence only.

- **Due to significant societal impact of CRC, lay public may be educated towards moderate sun exposure** whilst remaining **alert on suspect skin lesions** that need to be periodically checked
  - » *...this may also mean better education of primary care physicians, but such recommendations need to be first adopted by professional societies*

thank you for your attention

- Lenka Zdrazilova Dubska
- Kristina Greplova
- Iveta Selingerova
- Vlad Popovici
- Radek Pilny
- Regina Demlova
- Ladislav Dusek
- Vit Dusek
- Radka Obermannova
- Jiri Jarkovsky
- Rostislav Vyzula
- Rudolf Nenutil