Detection of CTCs in the peripheral blood of patients with untreated SCLC using both the CellSearch platform and IFAT (TTF1, CD56).

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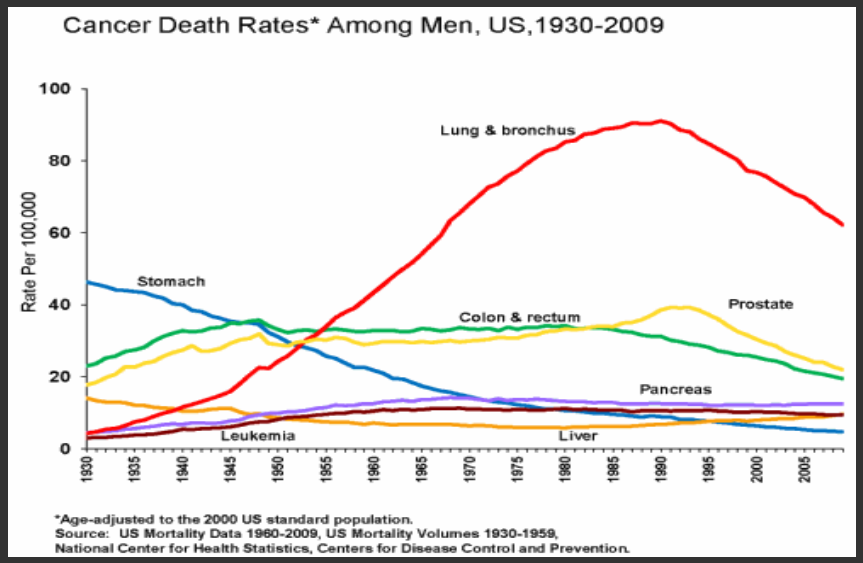
Background

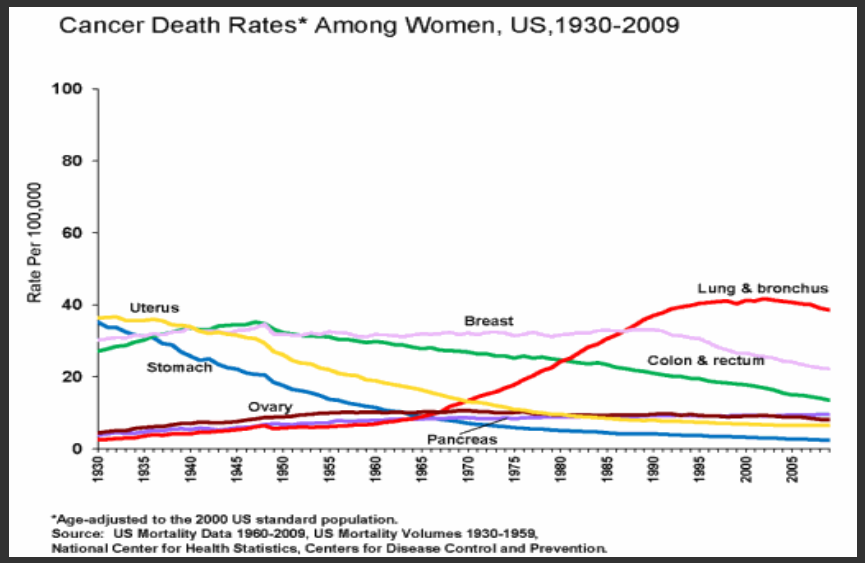
- Most pulmonary carcinomas are readily classified, based on morphological and nuclear features, into:
  - SCLC
  - NSCLC

- WHO:
  - >1.2 million new cases of lung and bronchial cancer diagnosed each year, worldwide
  - ~1.1 million deaths annually

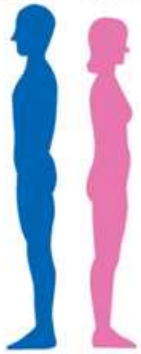
Background

- **Leading cause of cancer deaths in the US**
  - ~160.440 deaths in 2004,
  - 32% and 25% of all cancer deaths in men and women, respectively
- **- median age at diagnosis is ~70 yrs**





Males			Females		
Lung & bronchus	87,260	28%	Lung & bronchus	72,220	26%
Prostate	29,720	10%	Breast	39,620	14%
Colorectum	26,300	9%	Colorectum	24,530	9%
Pancreas	19,480	6%	Pancreas	18,980	7%
Liver & intrahepatic bile duct	14,890	5%	Ovary	14,030	5%
Leukemia	13,660	4%	Leukemia	10,060	4%
Esophagus	12,220	4%	Non-Hodgkin lymphoma	8,430	3%
Urinary bladder	10,820	4%	Uterine corpus	8,190	3%
Non-Hodgkin lymphoma	10,590	3%	Liver & intrahepatic bile duct	6,780	2%
Kidney & renal pelvis	8,780	3%	Brain & other nervous system	6,150	2%
All Sites	**306,920**	**100%**	**All Sites**	**273,430**	**100%**

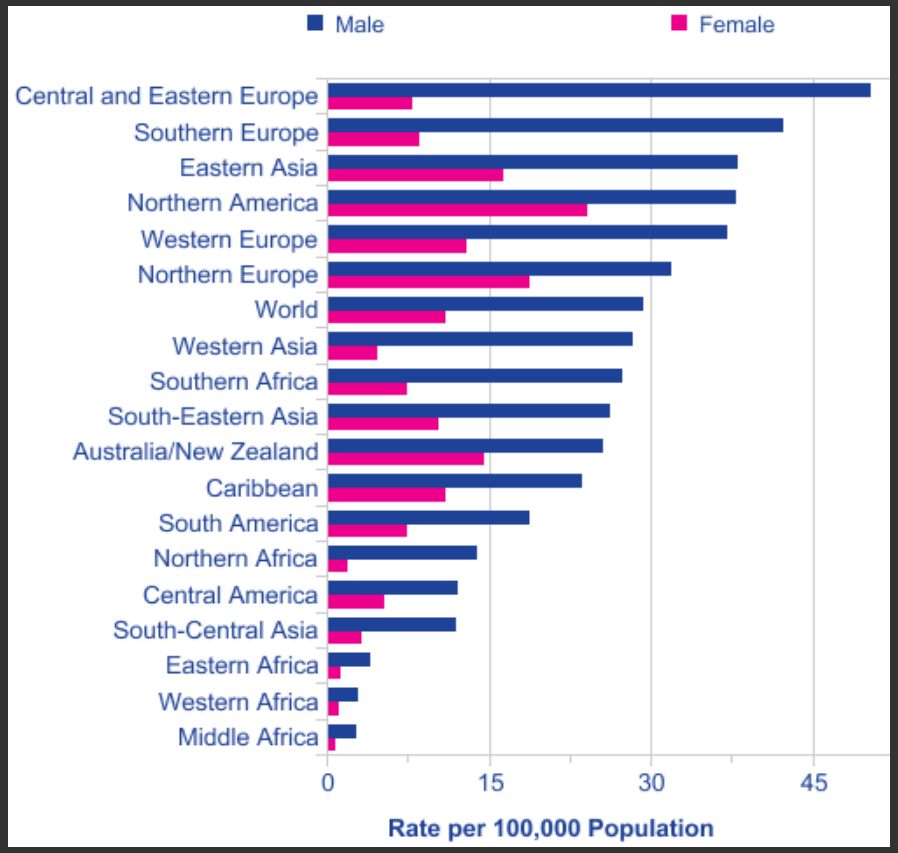


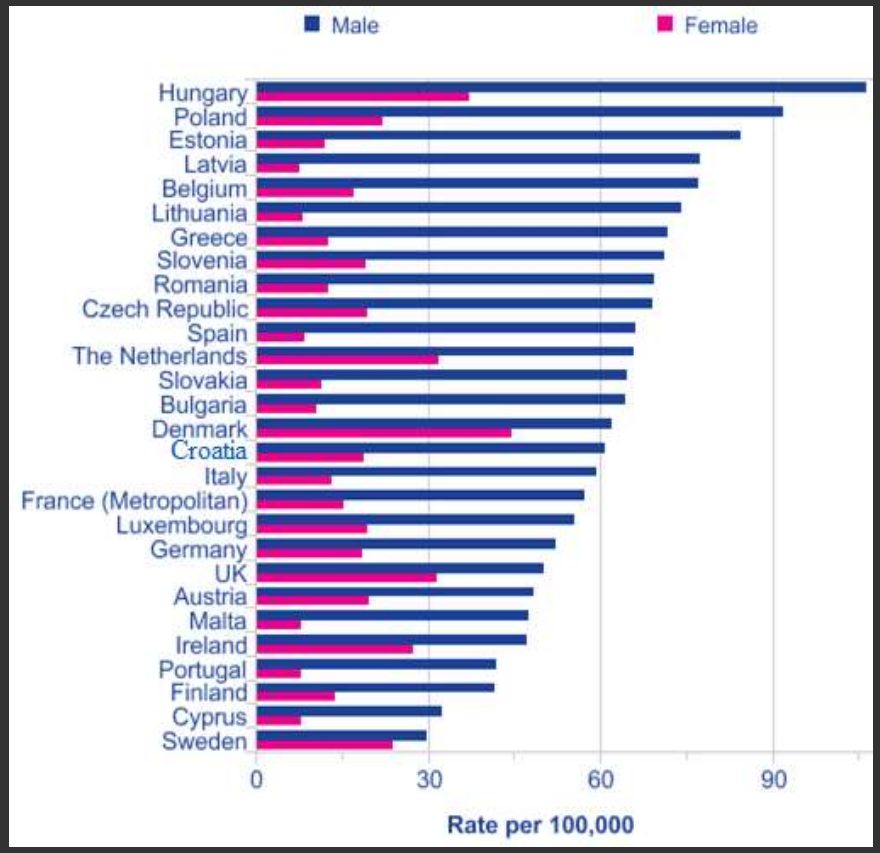
Siegel R, et al. Cancer Statistics 2013. *CA Cancer J Clin* 2013

Background

- In Europe,
  - ~400.000 new cases of lung and bronchial cancer diagnosed each year

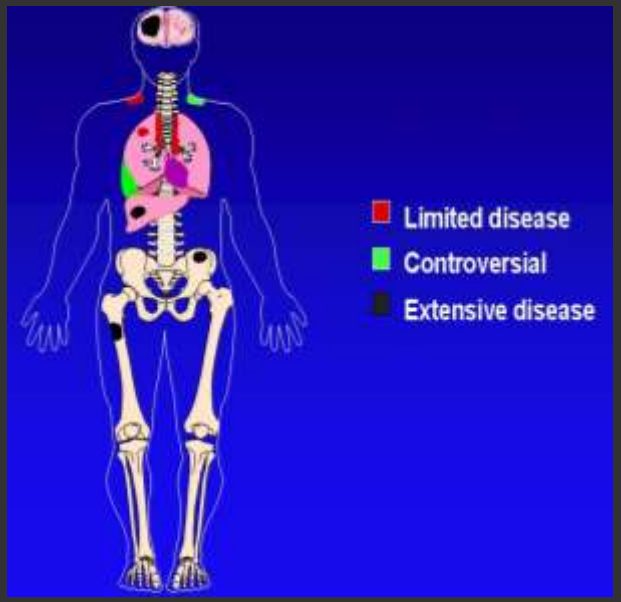
Bray F, et al. Eur J Cancer. 2002





Ferlay J, et al. GLOBOCAN 2008 v1.2. International Agency for Research on Cancer; 2010 Estimates of 2008

SCLC



1. ~15% of all lung cancer cases
2. Almost exclusively in smokers
3. Rapid doubling time
4. Early development of widespread metastases
5. High growth fraction
6. Highly responsive to chemotherapy and radiotherapy
7. Common relapses within short term, despite treatment
8. 20-40% of patients with limited disease survives > 5 years
9. 3-8% of patients with extensive disease survives > 5 years
10. Often is correlated with paraneoplastic syndromes (SIADH, Cushing's, Eaton-Lambert etc)
11. Blood spread on diagnosis

SCLC

- Morphological data to confirm the diagnosis of SCLC are increasingly supported by IHC using cytokeratins and neuroendocrine markers such as:
  - chromogranin A
  - CD57 (Leu-7)
  - synaptophysin
  - neurone specific enolase
  - CD56

- CD56:
  - antibody of choice in many laboratories
  - comparative studies suggest that CD56 is the most sensitive marker in this context

Kontogianni K, et al. J Clin Pathol 2005

- TTF-1:
  - high sensitivity for pulmonary SCLC

Ordenez NG. Am J Surg Pathol 2000

Purpose

Evaluation of the detection of CTCs in untreated patients with SCLC and after 1 cycle of chemotherapy, using the CellSearch assay, as well as the CD56 and TTF-1 detection markers

Materials and Methods

- IFAT → TTF-1, CD56

  - TTF-1: Anti-Thyroid Transcription Factor 1. A protein that regulates transcription of genes specific for the thyroid, lung, and diencephalon. It is also known as thyroid specific enhancer binding protein.

  - CD56: Neural Cell Adhesion Molecule. A homophilic binding glycoprotein expressed on the surface of neurons, glia, skeletal muscle and natural killer cells.

- CellSearch → EpCam +ve, CK 8/18 or/and 19

- RT-qPCR → TTF-1, under validation

Materials and Methods

SCLC
65 patients

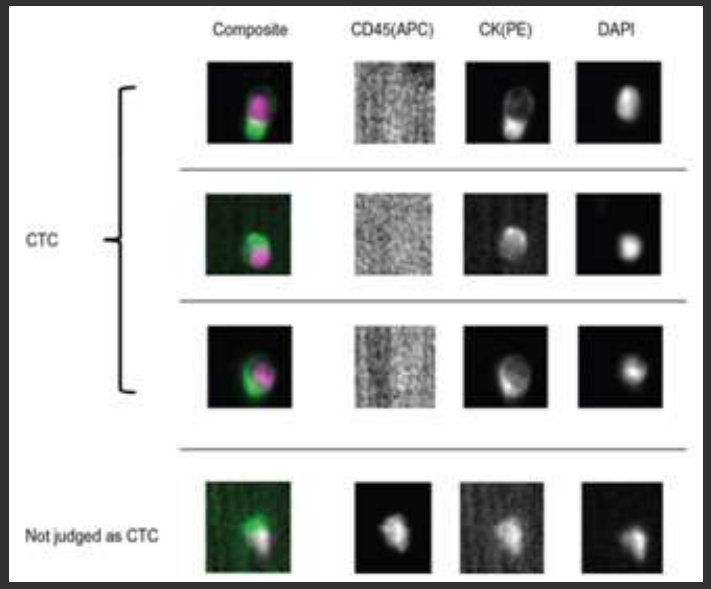
- **IFAT**
  TTF-1/CD56/DAPI
- **RT-qPCR**
  TTF-1
- **CellSearch**

10^6 PBMCs + 1 H209 (TTF-1)

10^6 PBMCs + 1 H209 (CD56)

10^6 PBMCs + 1 H209 (DAPI)

10^6 PBMCs + 1 H209 (Overlay)



Patients Characteristics

Feature	N (65)	%
Age		
Median	66,5 (44-82)	
Gender		
Male	56	86,2
Female	9	13,8
PS		
0-1	33	51,6
≥2	32	48,4
LD_ED		
LD	21	32,8
ED	43	67,2
Respose		
PR	45	69,2
SD	1	1,5
PD	14	21,5
NOT EVAL	5	7,7

Results at Baseline

	TTF1 pre chemo (Range 1-76)		
	N (65)	%	mean (SD)
ND	1	1,5	
pos	27	41,5	4,38 (12,14)
neg	37	56,9	

	CD56 pre chemo (Range 1-940)		
	N (65)	%	mean (SD)
ND	1	1	
pos	18	28,1	32,4 (140,60)
neg	46	71,9	

	TTF1_CD56 pre chemo (Range 1-18)		
	N (65)	%	mean (SD)
ND	1	1,5	
pos	8	12,3	1,0 (3,46)
neg	56	86,2	

	CellSearch pre chemo (Range 1-10000)		
	N (65)	%	mean (SD)
ND	26	39,1	
pos	30	46,9	745,44 (2050,1)
neg	9	13,8	

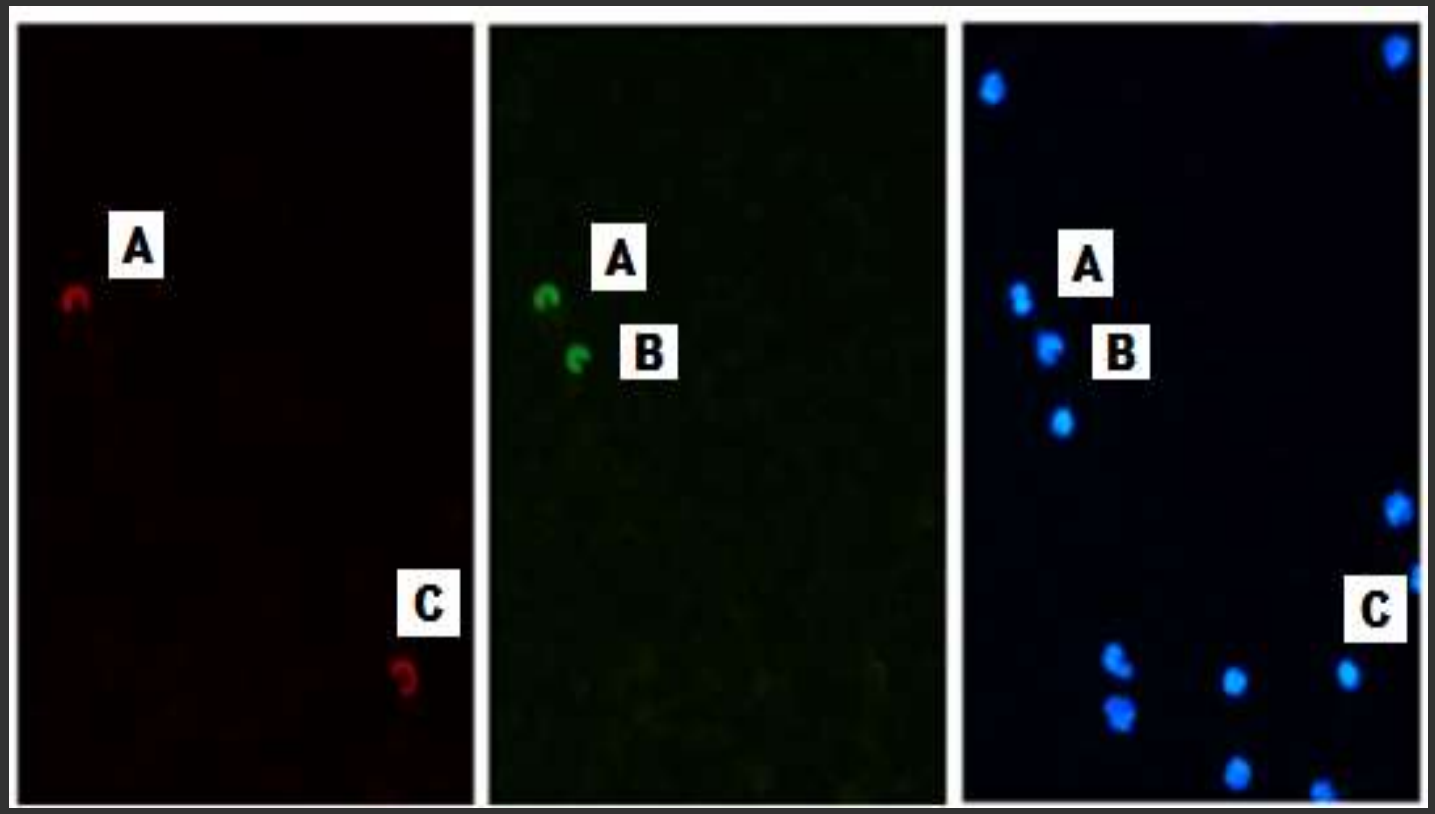
50% of the patients: TTF1 and/or CD56 positive CTCs

76% of the patients: IFAT and/or CellSearch positive CTCs

26% of the patients: CellSearch (only) positive

23% of the patients: IFAT and CellSearch negative

Results



CD56 TTF-1 DAPI

Results post chemo

	TTF1 post chemo (Range 1-40)		
	N (65)	%	mean (SD)
ND	18	27,7	
pos	15	23,0	3,55 (8,90)
neg	32	49,2	

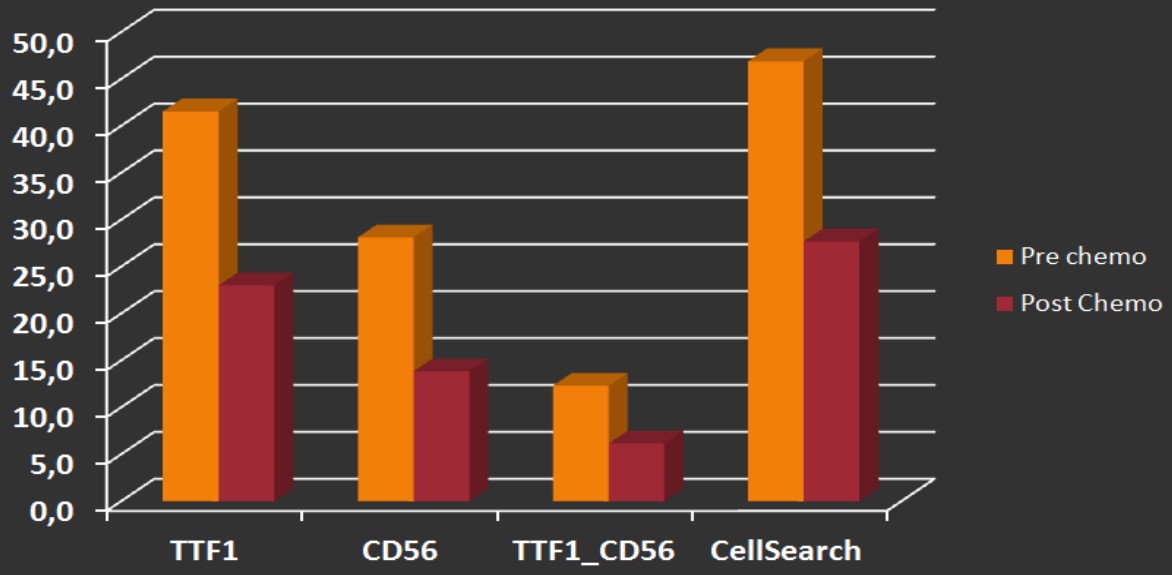
	CD56 post chemo (Range 1-107)		
	N (65)	%	mean (SD)
ND	18	27,7	
pos	9	13,8	6,47 (19,69)
neg	38	58,5	

	TTF1_CD56 post chemo (Range 1-8)		
	N (65)	%	mean (SD)
ND	18	27,7	
pos	4	6,2	0,36 (1,40)
neg	43	66,2	

	CellSearch post chemo (Range 1-4882)		
	N (65)	%	mean (SD)
ND	26	40,0	
pos	18	27,7	292,51 (973,64)
neg	21	32,3	

Results

	Pre chemo	Post chemo
	Positivity % (Range of CTCs)	
TTF1	41,5 (1-76)	23,0 (1-40)
CD56	28,1 (1-940)	13,8 (1-107)
TTF1_CD56	12,3 (1-18)	6,2 (1-8)
CellSearch	46,9 (1-10000)	27,7 (1-4882)



Conclusions

- Detection of CTCs using either IFAT or CellSearch may be used as a surrogate marker for the efficacy of systemic treatment.

- The method merits further evaluation in larger patients cohorts

Future plans

- Comparison of the sensitivity and specificity of the methodologies with molecular methods (RT-qPCR)
- Correlation with clinico-pathological parameters

- To investigate the monoclonal antibody M30 for the assessment of apoptosis
- To assess tumor proliferation by Ki67
- Assessment of CD133 expression
- Assessment of ALDH expression