



Dendritic Cell Recruiters and Activators

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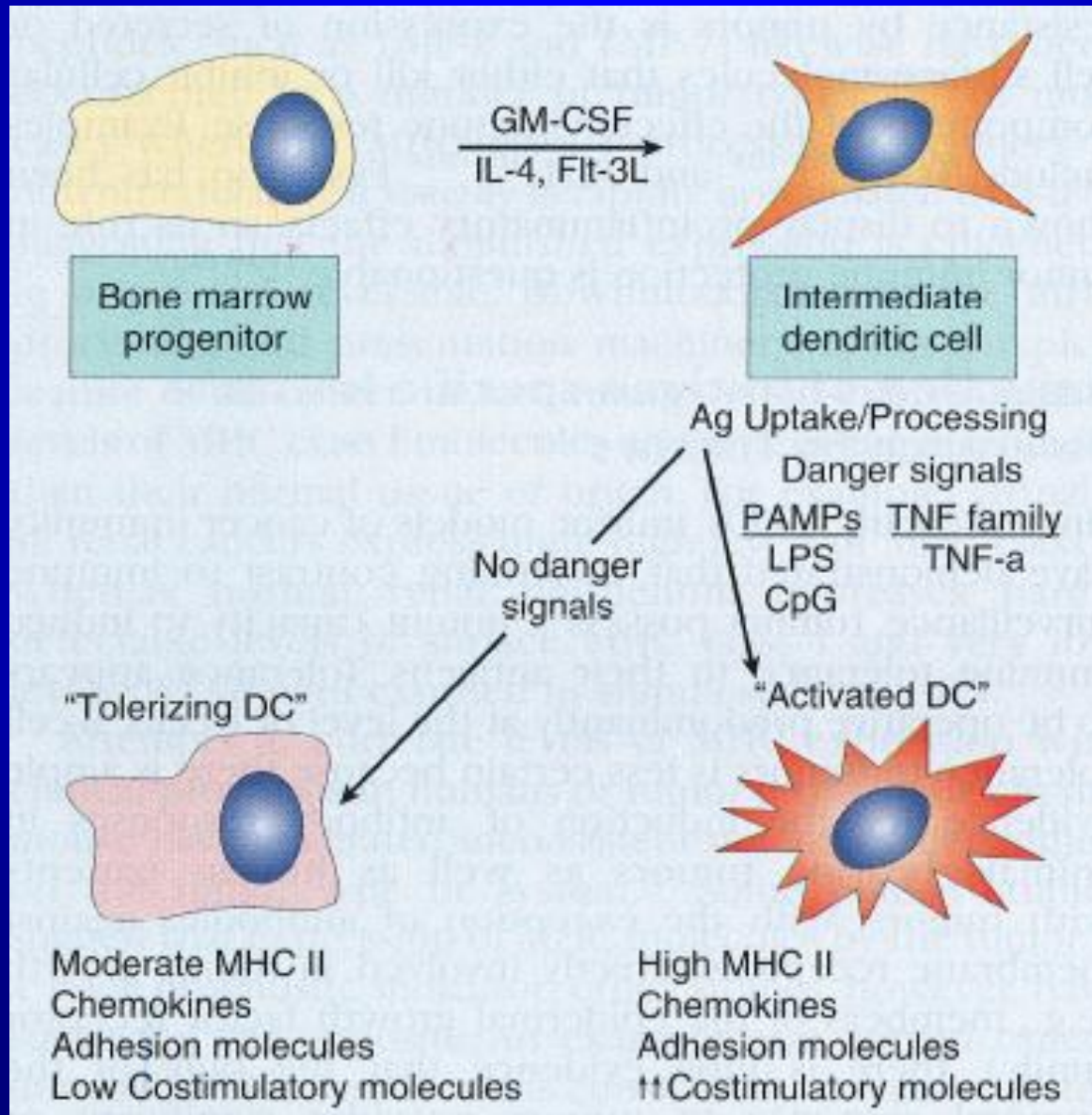
Dendritic Cells (DC)

- Most effective antigen-presenting cells for initiating naïve T-cell response. They stimulate primary immune response. They also support tolerance to self-antigen
- Activation or tolerance, two conflicting activities appear to relate to different stage of maturation of DC
- Activated DC travel to regional lymph node to stimulate NK and NK T cells and the production of antigen specific cytotoxic T cells

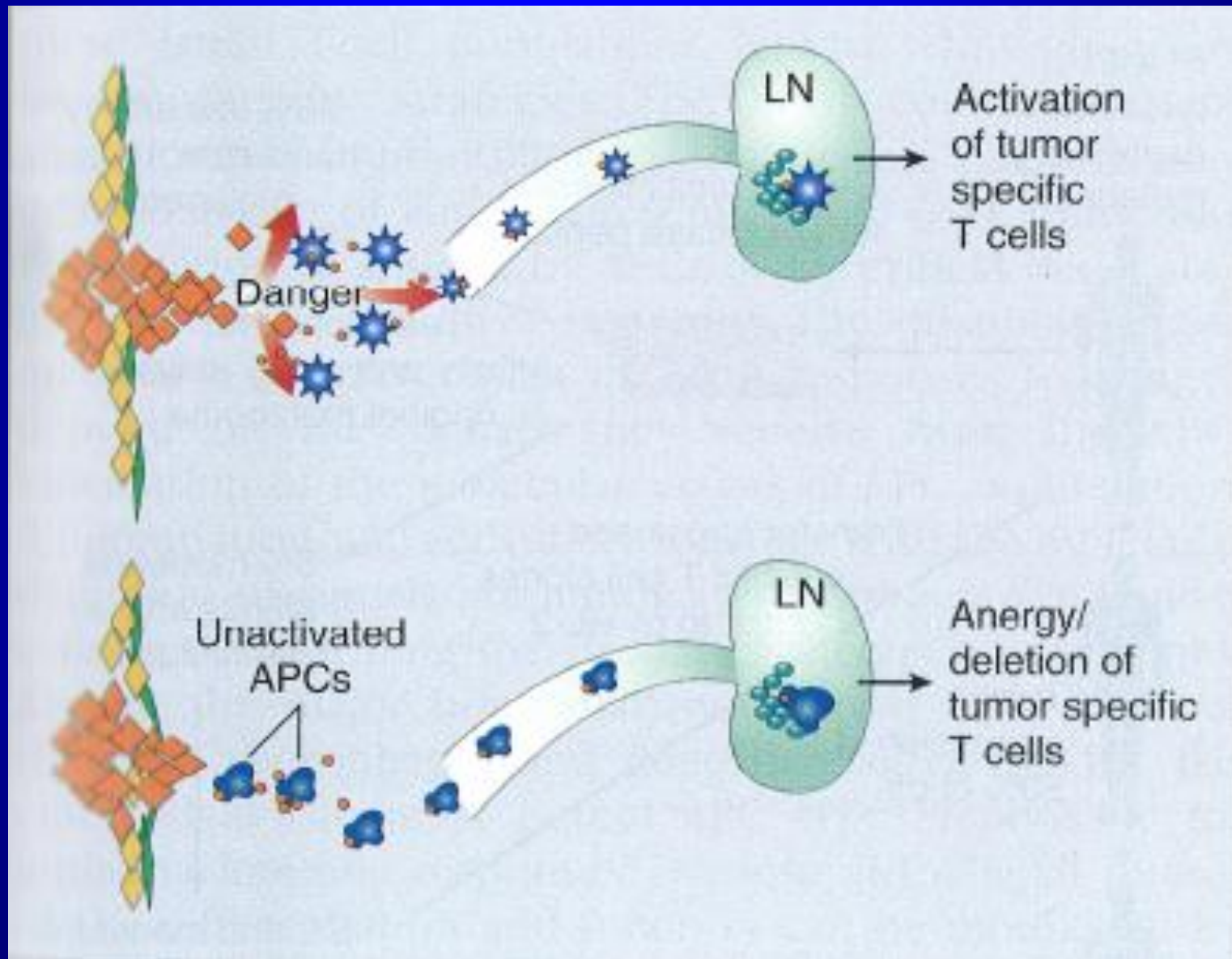
Origins of Dendritic Cells

- Myeloid precursors (monocytes, CD14+)
- Plasmacytoid
- Langerhans cells (in the epidermis of skin and epithelium of mucosa)
- CD34+ stem cells

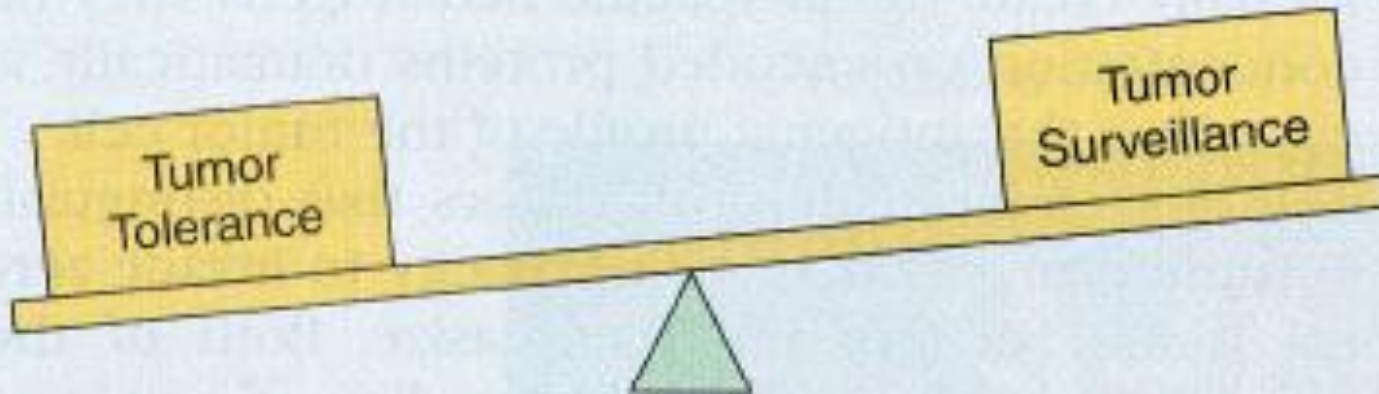
Pathways of Dendritic cell Differentiation



Potential Immunologic Outcomes of Tumor Development



Tumor Tolerance vs Tumor Surveillance



Tolerance

- Tolerance generation in many tumor models
- In general, hard to grow T cells against tumors
- TCR-pep/MHC affinities for tumor Ag typically low

vs.

Surveillance

- Immune activation in some tumor models
 - Concomitant immunity
 - "Regressor" tumors
- Increased tumor incidence in immunodeficient mice

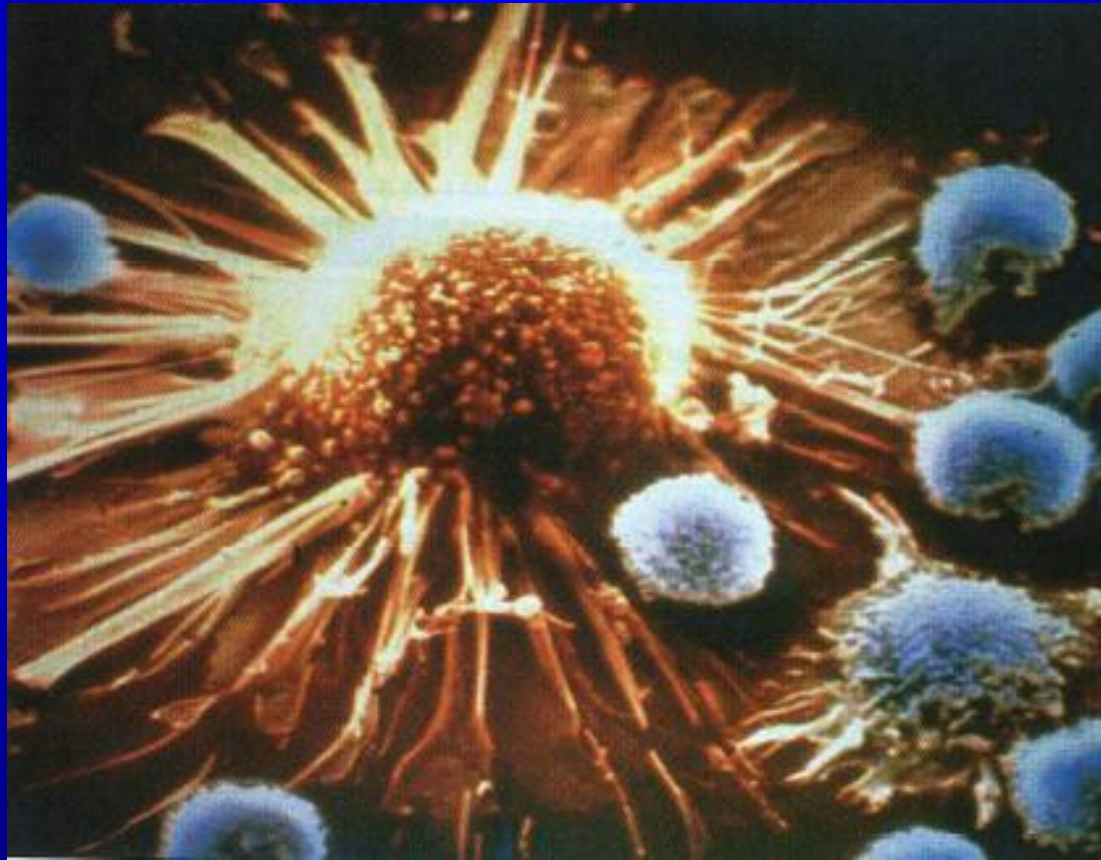
Mechanisms of Immune Tolerance by DC

- Antigen presentation with insufficient co-stimulation
- DC tryptophan metabolism resulting in either toxic to bound T cells or insufficient level to sustain T cell proliferation
- Induction of several different population of regulatory T cells – such as CD4+/CD25+ that suppress the activities of other T cells (suppressor T cells)

Down regulation of Dendritic Cells (DC) by Cancer Cells

- Cancer cells promote altered maturation in DCs with, decreased capacity to produce IL-12 and IL-10. (Kiertcher SM et al, J Immunol 2000; 164:1269-1276)
- Both circulating and tumor infiltrating DCs are functionally impaired in cancer patients
- Tumor secreted vascular endothelial growth factor (VEGF), M-CSF, IL-6, etc. inhibit NF-KB activation and DC acquisition of CD80, CD86 and HLA-DR. Net result : Minimum recruitment and activation of dendritic cells in cancer mass. (Troy AJ. et al. Clinical Ca Res 1998; 4:585)

Tumor specific cytotoxic T Lymphocyte



Dendritic Cells – Yin and Yang

Tumor cells

Cyclo-oxygenase 2

Ceramide

IL-10

TGF- β

Prostaglandin E2



IL-4

GM-CSF

IL18

Flt 3

PU1

TNF

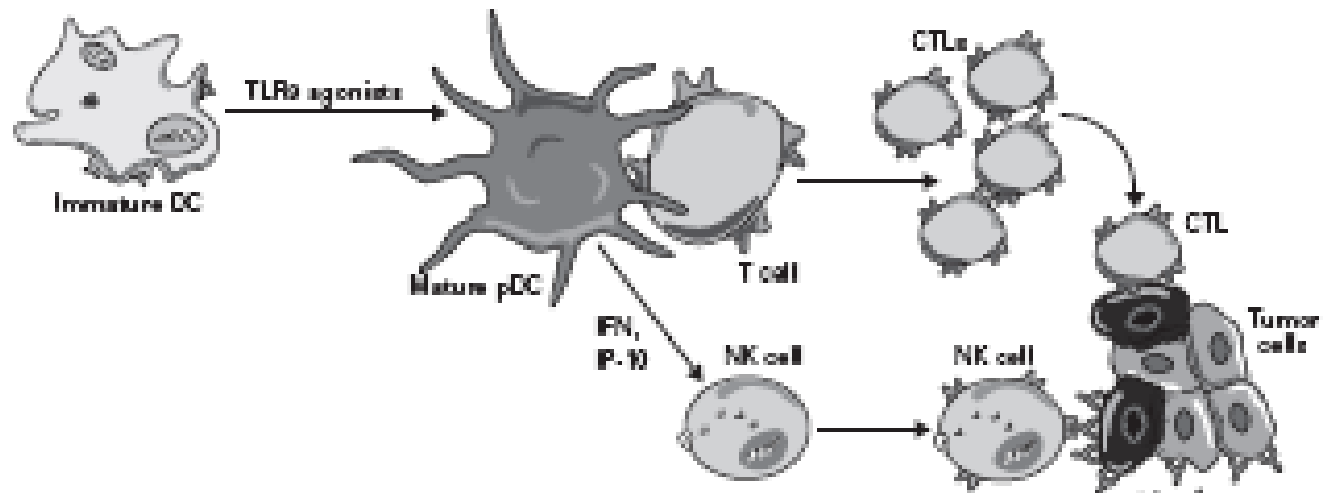
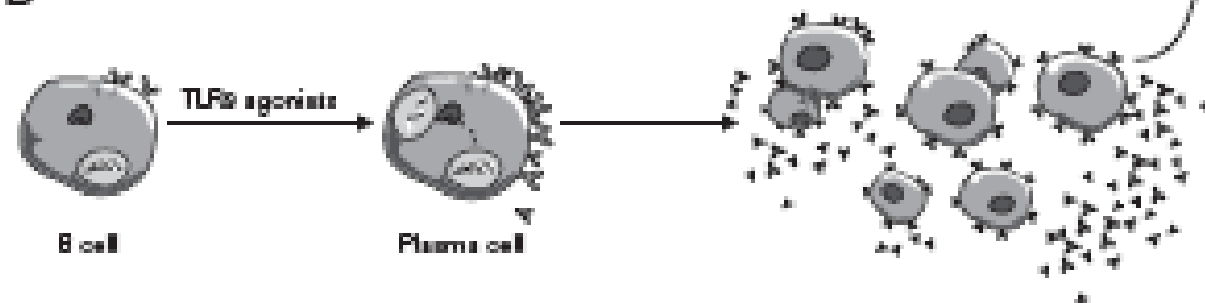
IL15

Strategies for Employing DC for Cancer Therapy

- DC recruiters and activators
- DC cellular therapy
- DC vaccine

PF 3512676 (Pro Mune, agatolimod) contains unmethylated cytosine and guanine (CpG) motif and a nuclease-resistant Phosphorothioate backbone

- It increases expression of plasmacytoid DCs of MHC I & II and co-stimulatory molecules to enhance antigen presentation, pDCs secrete cytokines and chemokines that stimulate NK cells, and generate long-living antigen specific cytotoxic T cells and antibody response
- PF3512676 increases the production of IFN- α and interferon-inducible protein 10 (IP-10), which is an anti-angiogenic cytokine, in DCs. It also upregulates CD86 and CD80 and increases secretion of IL-10, IL-6 in B lymphocytes

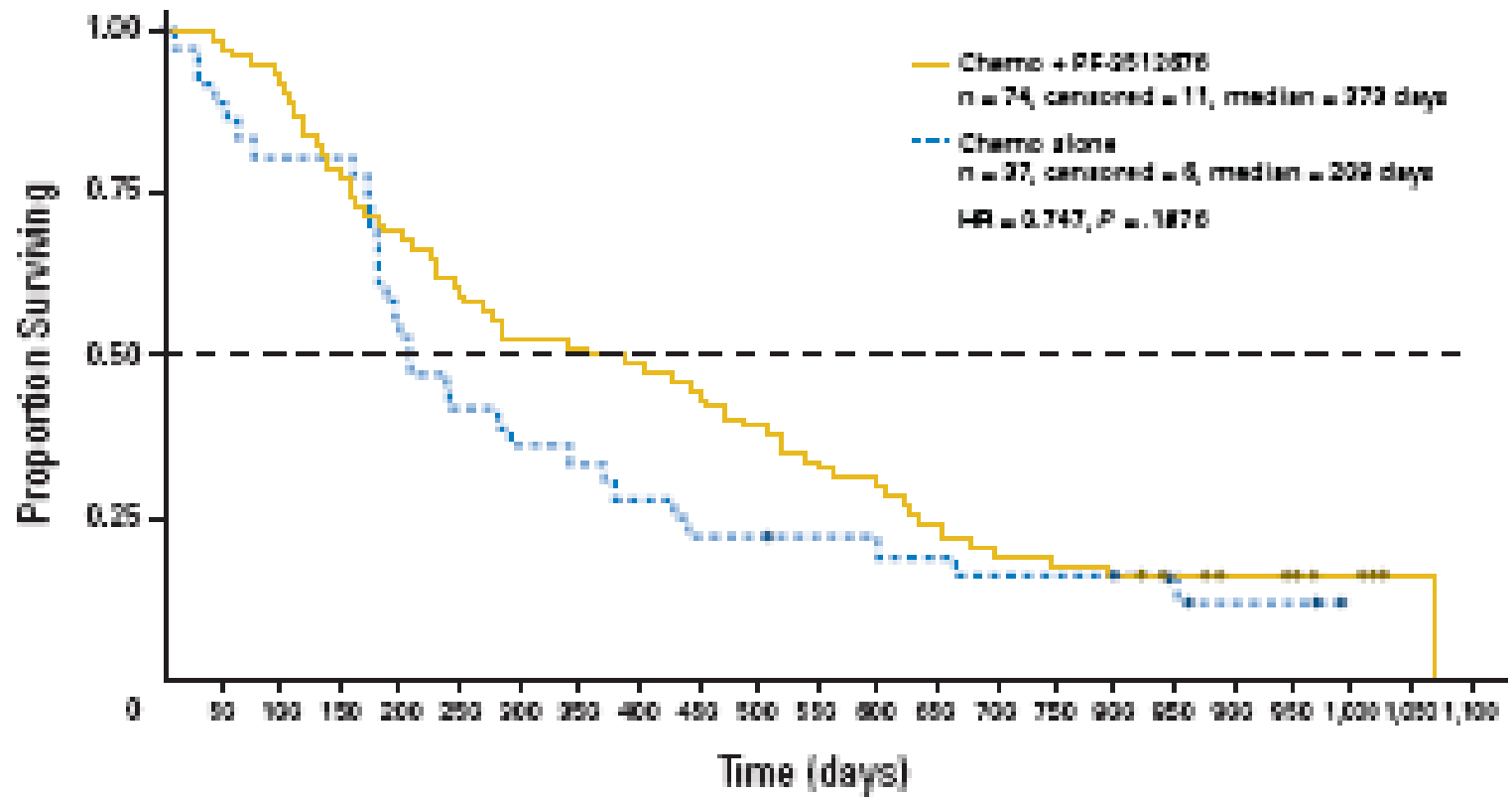
A**B**

Randomised Phase II Trial of Taxane + platinum ± PF3512676 in 1st line therapy for Advanced NSCLC

	N	ORR	MS	1 YSR
T + P day 1 q3wk	34	19% (11%)	6.8m	33%
T + P + PF (0.2mg/kg S/Q d 8,15) q3wk	75	38% (19%) P=0.043	12.3m P=0.188	50%

Primary endpoint: ORR

Manegold C et al JCO 2008; 26: 3979-86



Phase III Randomised Trials Studying PF-3512676 in Combination with Chemotherapy in Advanced NSCLC

	N	ORR	PFS	MS
Paclitaxel/Carbo	429	23%	4.8	10.3
Paclitaxel/Carbo + PF	409	25%	4.8	10.2
<i>Hirsh et al JCO 2008; 26 (Suppl 15):428s</i>				
Cisplatin/Gem	423	27%	5.2	10.7
Cisplatin/Gem + PF	416	29%	5.1	11.1
<i>Manegold et al JCO 2008; 26 (suppl 15):428s</i>				

PF-3512676 in NSCLC

It met the primary endpoint in randomised phase II trial, but failed in the randomised phase III study

Talactoferrin Alfa

- **Talactoferrin alfa (TLF) is a unique recombinant human lactoferrin**
 - 80 kD protein produced in *Aspergillus niger* (*A. niger*)
- **Human lactoferrin is an important immunomodulatory protein**
 - It is expressed throughout the body in immune cells and on all body surfaces exposed to the external environment
 - Found in the highest concentrations in milk and colostrum
 - Plays a central role in helping establish the immune system, including the gut associated lymphoid tissue (GALT), in infants

Talactoferrin is a Targeted Dendritic Cell (DC) Recruiter and Activator

DCs play a critical role in activating both Innate and Adaptive Immunity

Talactoferrin, taken orally, acts on the GI epithelium to release key chemokines (e.g. CCL20)



Immature dendritic cells (iDCs) are recruited to the GALT by chemokines and undergo maturation/activation

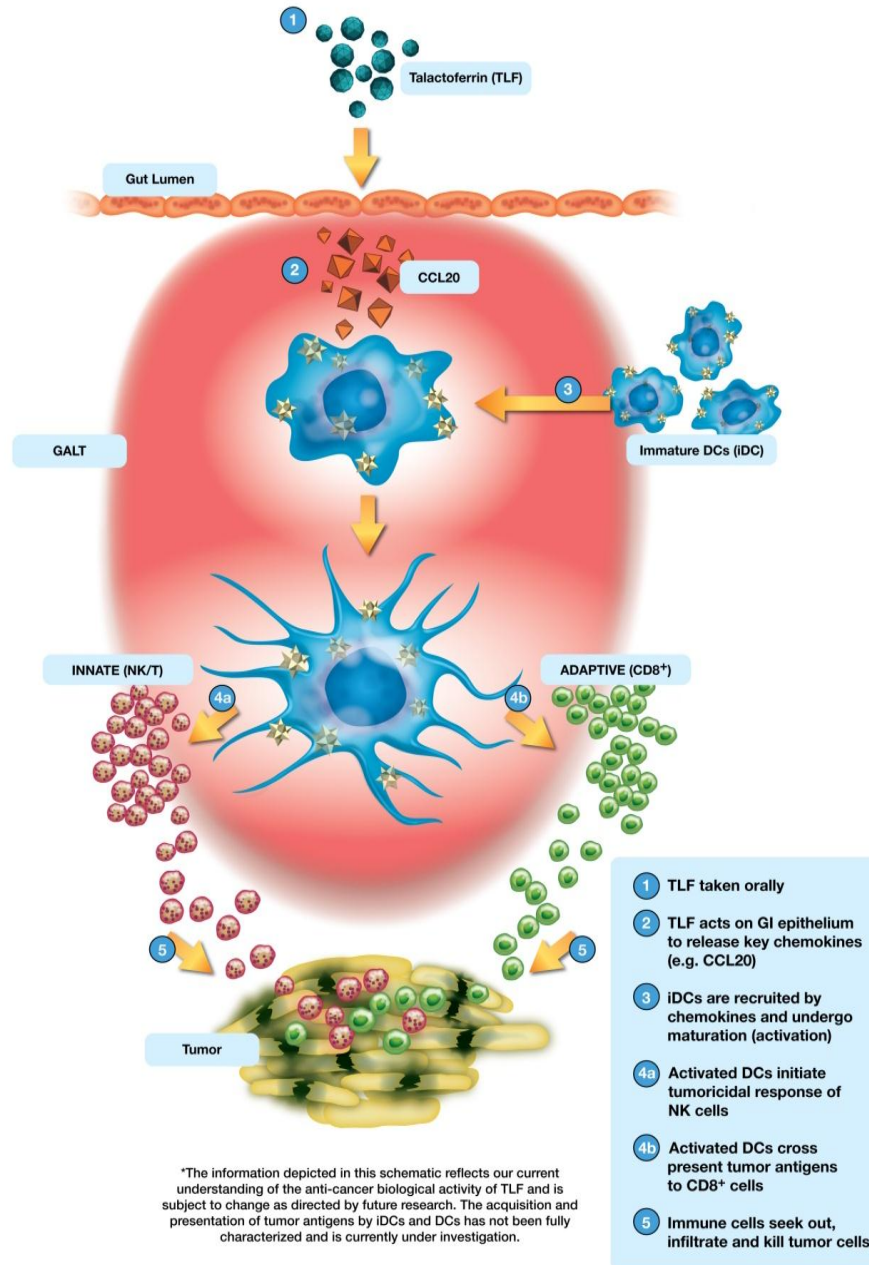


Activated dendritic cells initiate tumoricidal response of NK and NK-T cells (Innate immunity) and cross present tumor antigens to CD8+ lymphocytes (Adaptive immunity)



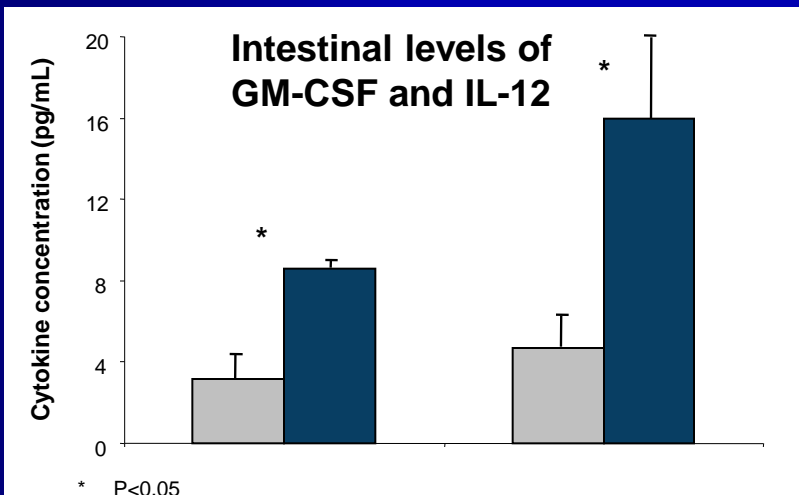
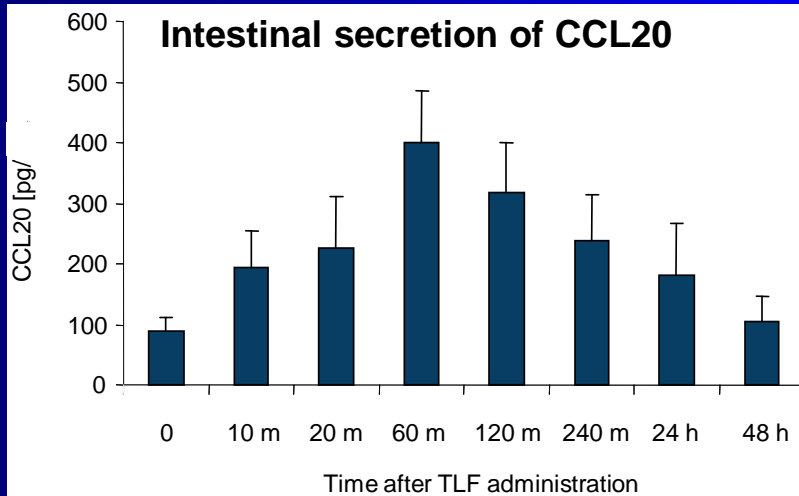
Immune cells seek out, infiltrate and kill tumor cells

Proposed Mechanism of Action for Talactoferrin*

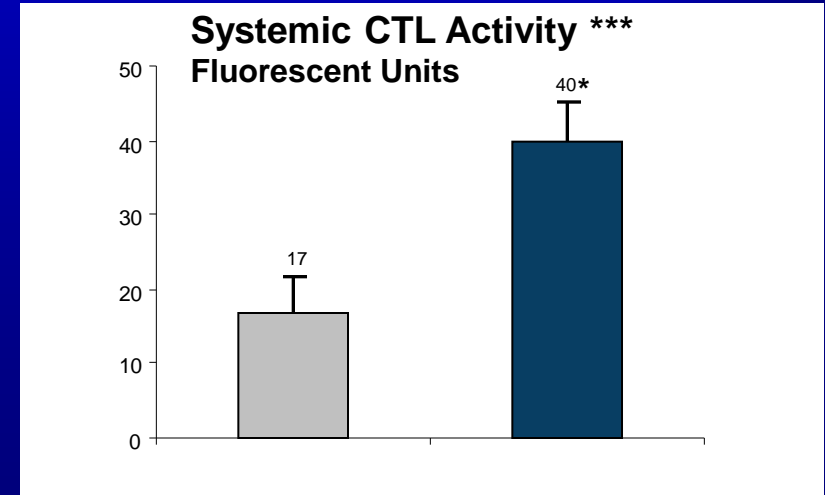
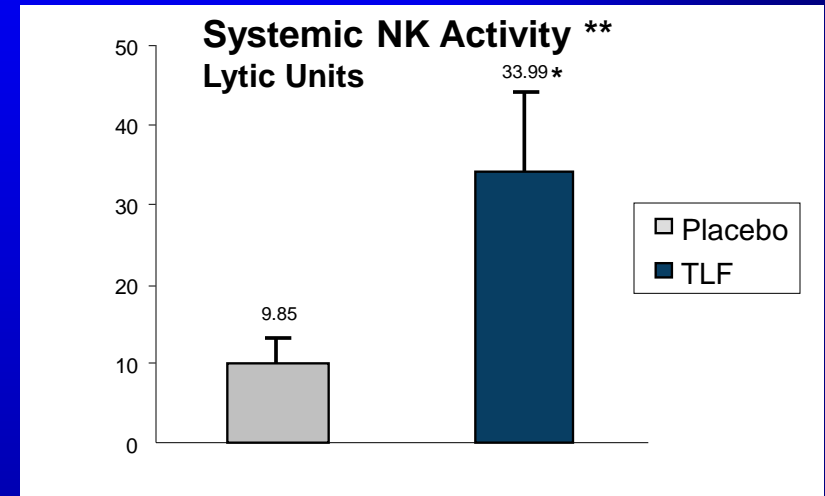


Oral Talactoferrin Activates Immune System

...in the GALT...



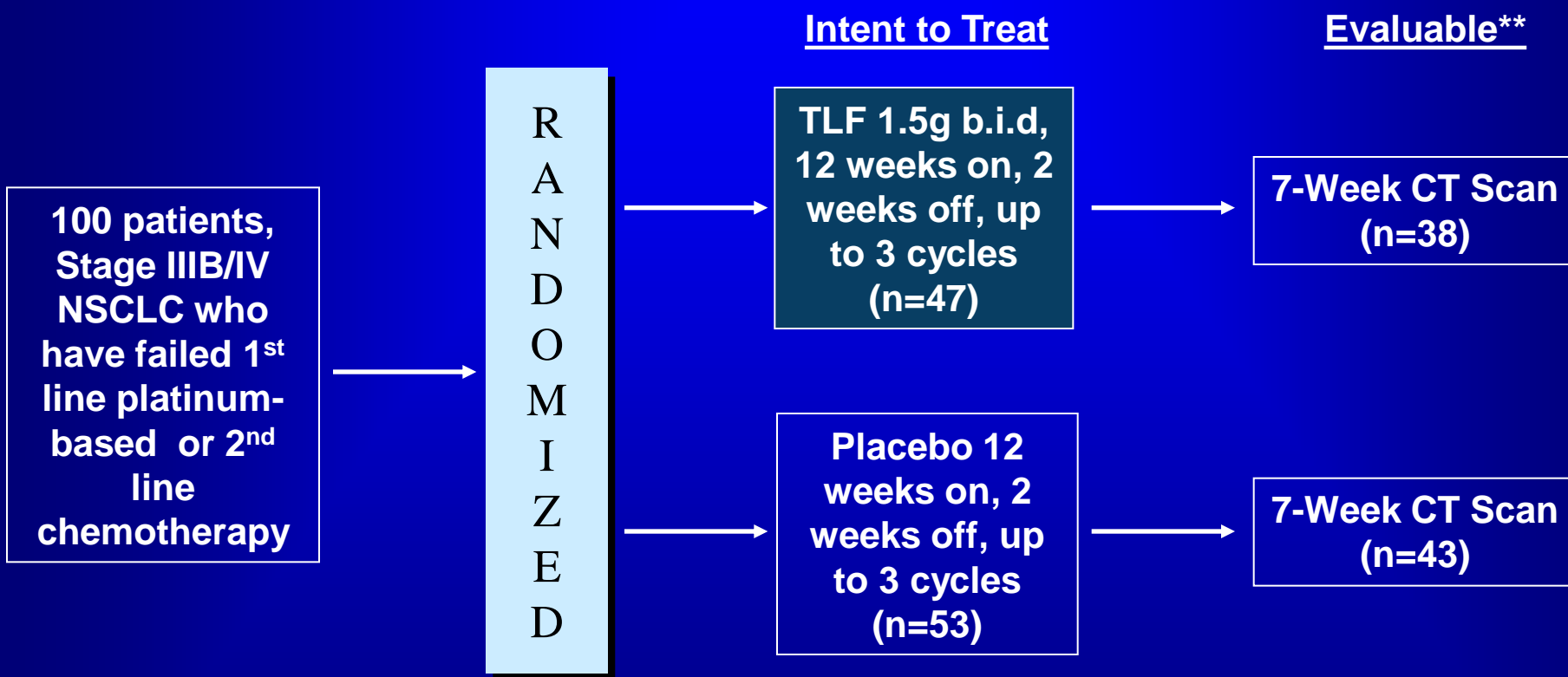
...and systemically



** Groups of 6 BALB/C naïve mice received placebo or TLF (0.9 g/m²) orally for three days. Twenty-four hours after the last dose, mice were sacrificed and spleens collected. NK cells were separated, pooled and tested *in vitro* for NK activity against YAC targets.

*** *In vivo* cytotoxicity was assessed in mice (8/group) treated with TLF (once a day for 2 cycles of 5 days/cycle) or placebo. The assay was performed as described by Ritchie et al. *J.Immunol.Methods*, 46; 109-117, 2000. In collaboration with Dr. Guido Forni, University of Turin.

LF-0201: Phase II Study of Talactoferrin (TLF) vs. Best Supportive Care (BSC) in Refractory NSCLC



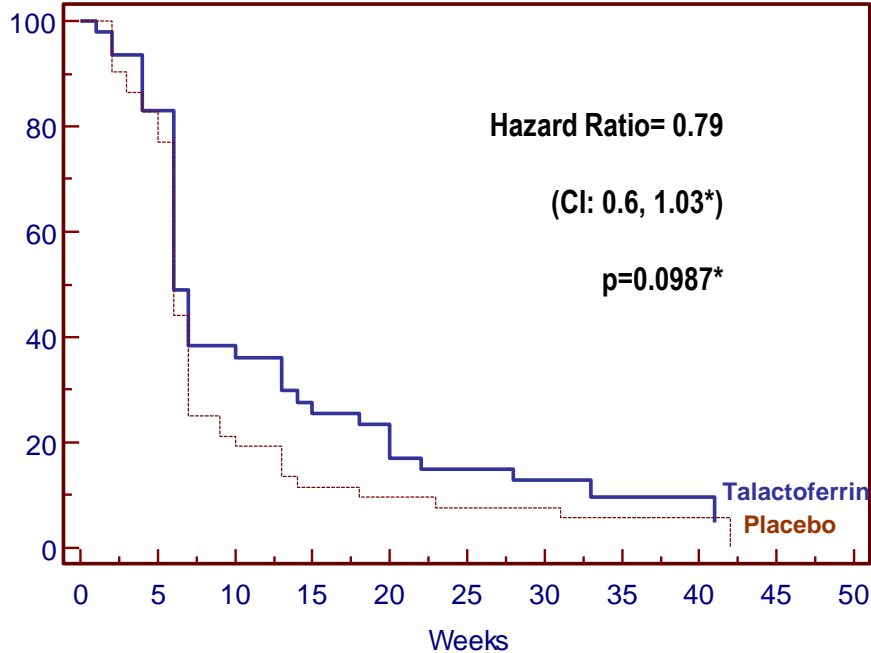
Primary Objective: Median Overall Survival, 6 month & 1 year OS (ITT population)

Secondary Objectives: Time to Progression, Tumor Response Rate, Toxicity & QoL (ITT & Evaluable populations)

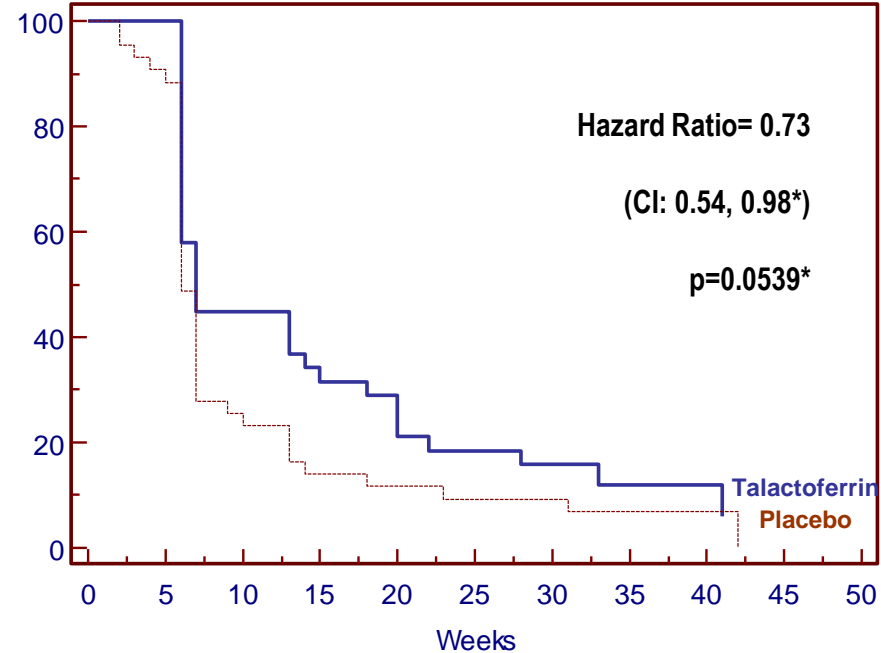
**Prospectively defined as patients who had at least one CT scan after start of therapy (~7 weeks)

LF-0201: Progression Free Survival

ITT Population



Evaluable Population



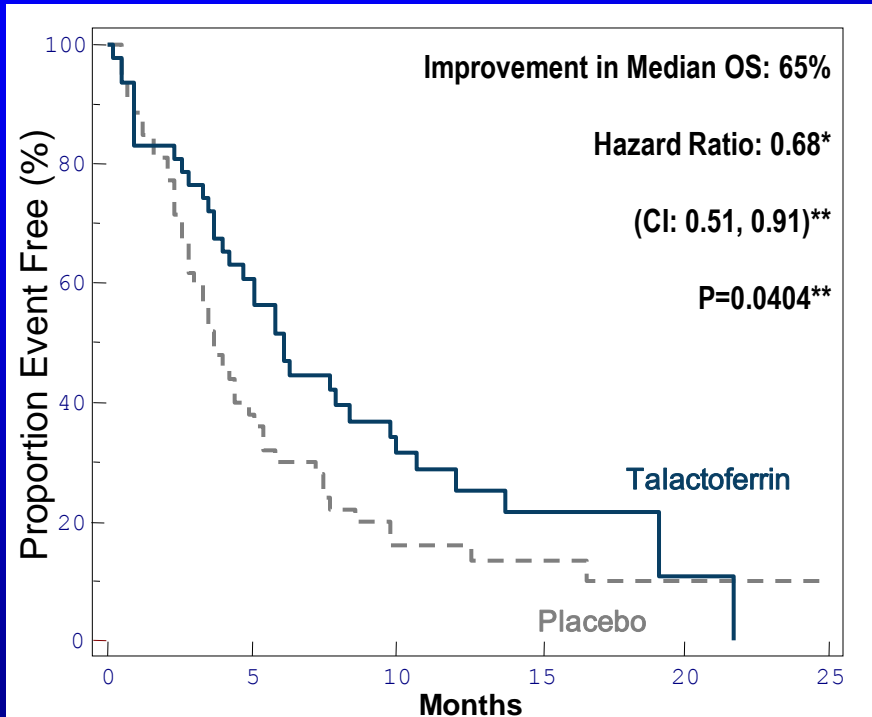
	0	5	10	15	20	25	30	35	40	45	50	
# patients at risk	47	18	11	5	2	0	38	17	11	5	2	0
	53	11	5	4	3	0	43	11	5	4	3	0

PFS was measured from the date of randomization to the date of tumor progression or death. Seven patients whose date of progression was not known were censored to the date of their last non-progression CT

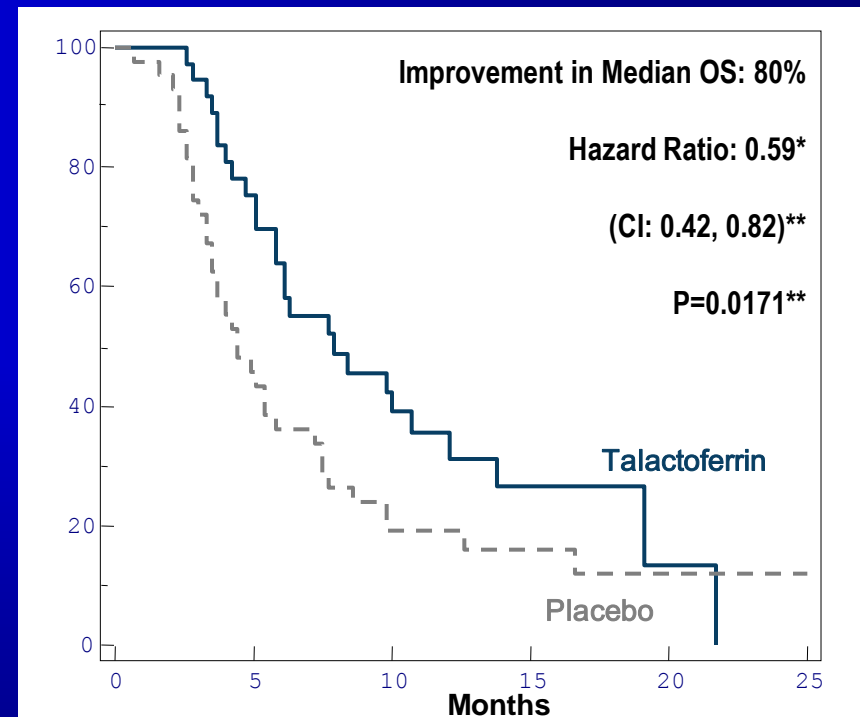
* One-tailed p-value using log-rank test; 90% CI as pre-specified in the protocol.

LF-0201: Improvement in Overall Survival

**ITT Population
(n=100)**



**Evaluable Population
(n=81)**



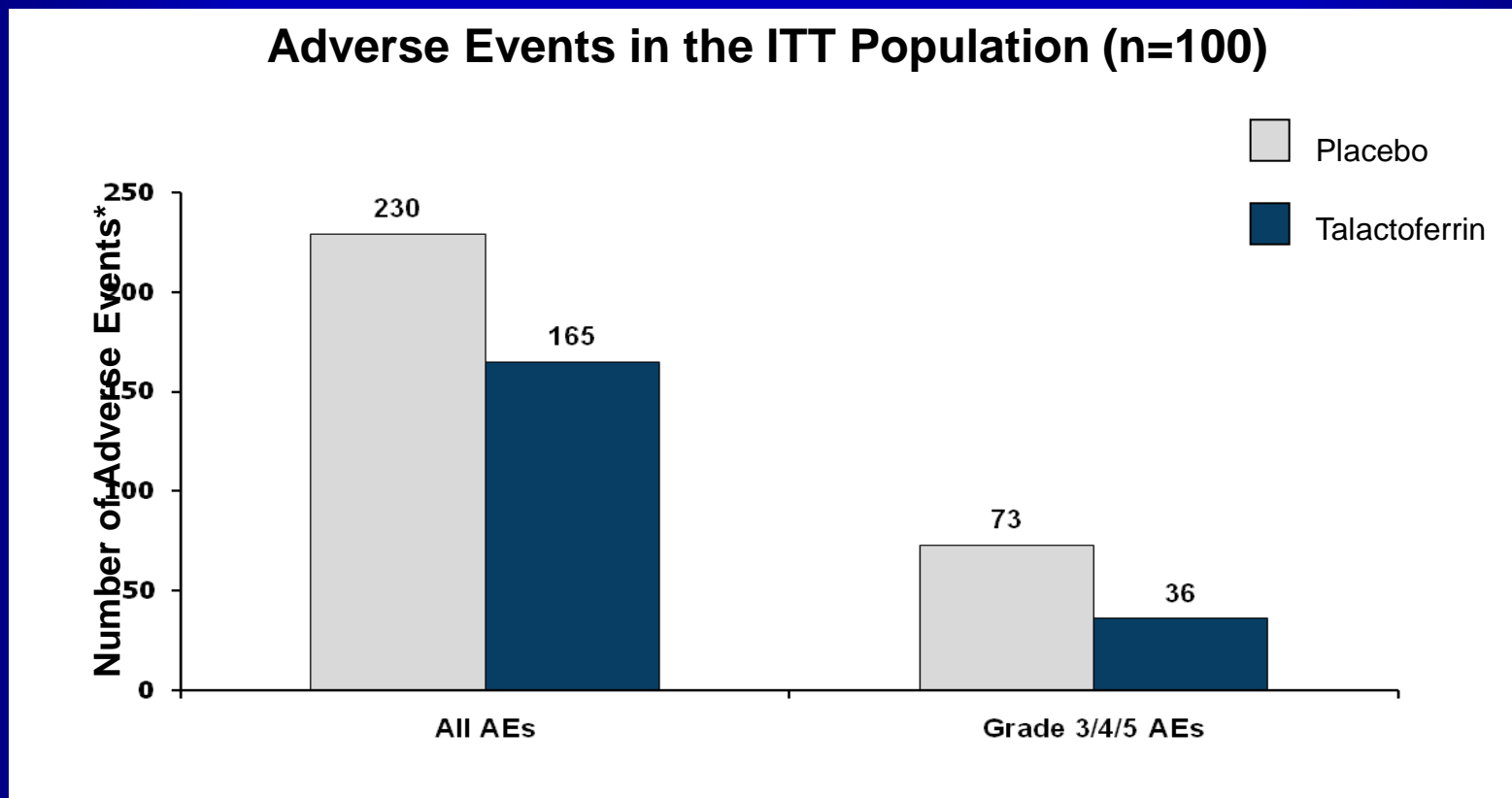
# patients	47	27	13	5	1	0
at risk	53	19	8	4	1	1

# patients	38	27	13	5	1	0
at risk	43	19	8	4	1	1

* The protocol-specified follow-up was for a maximum 18-months. 4 patients (2 in each arm) were followed beyond this. Results from analysis with patients censored at 18 months per protocol: HR = 0.64 (ITT) and 0.54 (Evaluable) with 1-tailed p-value = 0.023 and 0.008, respectively. The more conservative results are presented here.

** One-tailed p-value using log-rank test; 90% CI as pre-specified in the protocol.

LF-0201: Adverse Events



Improvement

28%

51%

P-value**

0.0013

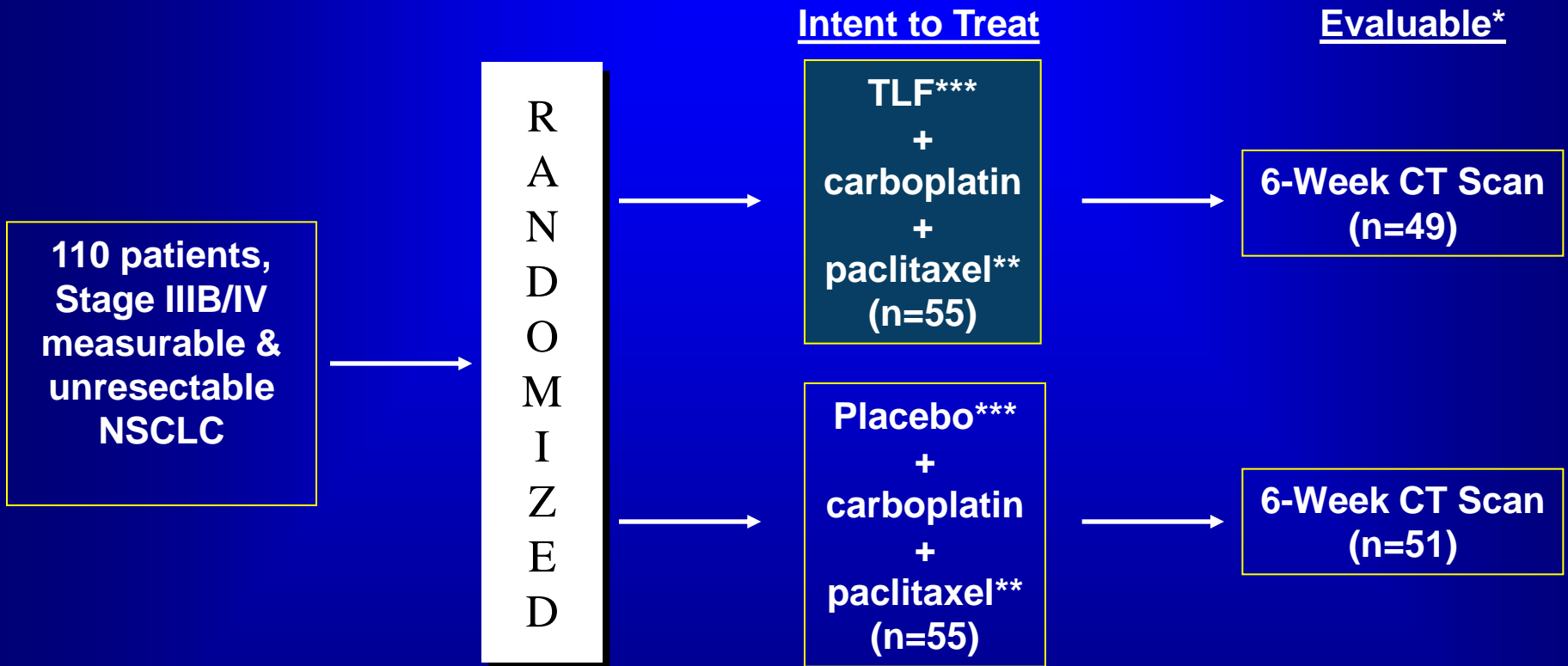
0.0005

* There were fewer patients in the talactoferrin arm, however this arm had a longer average duration of therapy and more total patient treatment weeks

** Two-tailed p-value obtained using Two-Proportion Binomial Test

ASCO 2007, Abstract #7540 updated

LF-0206: Phase II Study of Talactoferrin (TLF) in Combination with Carboplatin/Paclitaxel (C/P) in 1st Line NSCLC



Primary Endpoint: Confirmed Response Rate in Evaluable Patient Population

Secondary Endpoints: PFS, Overall Survival, 1-Year Survival and QoL

*Prospectively defined as patients who had at least one CT scan after start of therapy (~6 weeks)

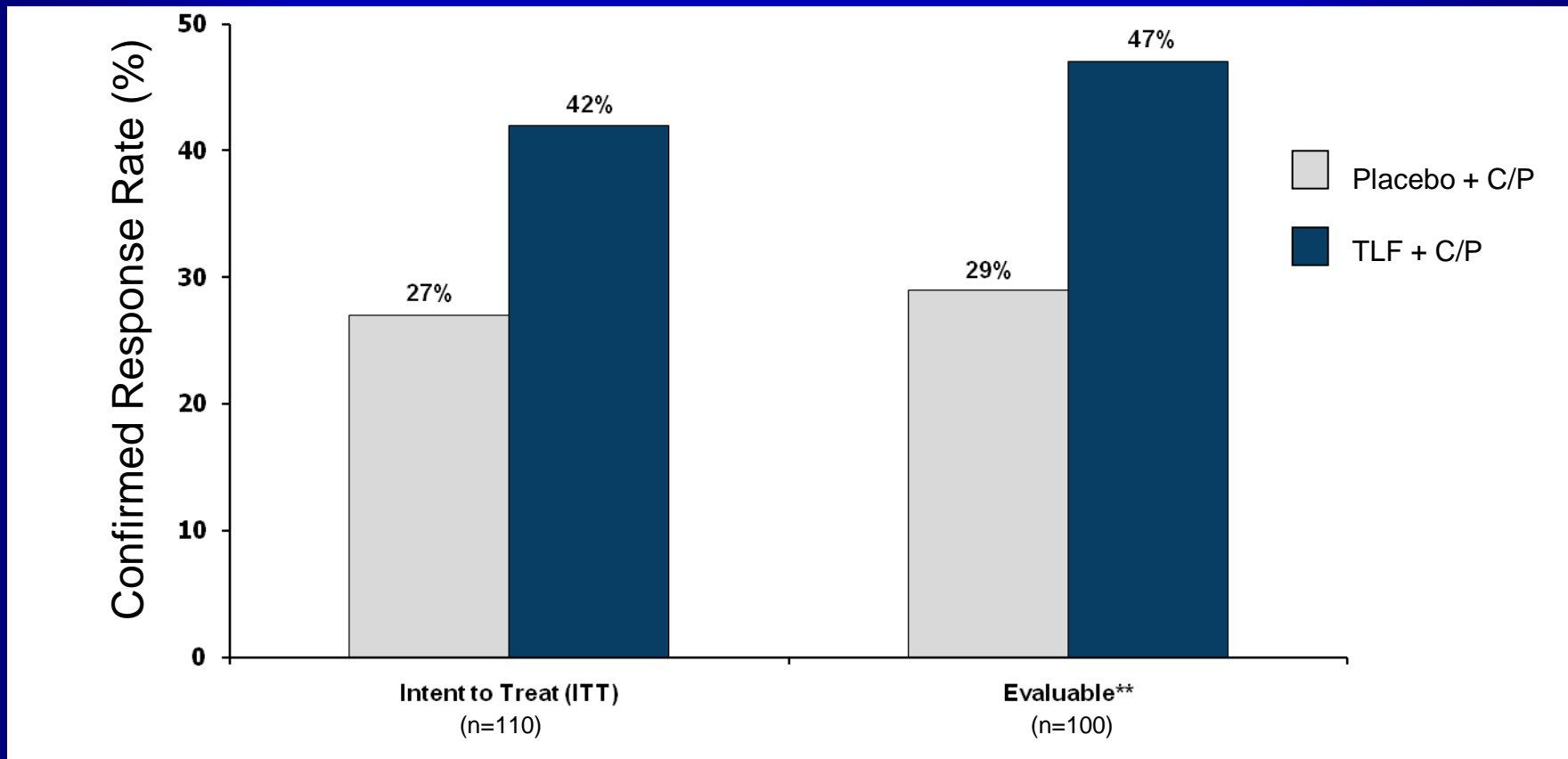
- ** Carboplatin (AUC 5 mg/mL/min) + Paclitaxel (175 mg/m²) in 3-week cycles

*** Placebo or Talactoferrin administered orally, 1.5 g B.I.D., in 35-day cycles for up to three cycles or until radiological progression, starting the day after C/P dosing in chemo-cycles 1, 3 and 5

LF-0206: Baseline Characteristics

Baseline Characteristic	<u>Talactoferrin + C/P</u>	<u>Placebo + C/P</u>
Number	55	55
Age (mean)	57.3	54.0
Disease Stage		
– IIIb	29%	38%
– IV	71%	62%
Performance Status		
– ECOG 0	16%	5%
– ECOG 1	84%	95%

LF-0206: Primary Endpoint of Confirmed Response Rate in Evaluable Population



Relative Improvement	56%	62%
P-value*	0.08	0.05

* P-values are one-tailed by Fisher's exact test. The trial protocol prospectively targeted a one-tailed p-value in the evaluable population of 0.05

** Defined as any patient who received at least one dose of talactoferrin/placebo, one dose of C/P and at least one post treatment CT

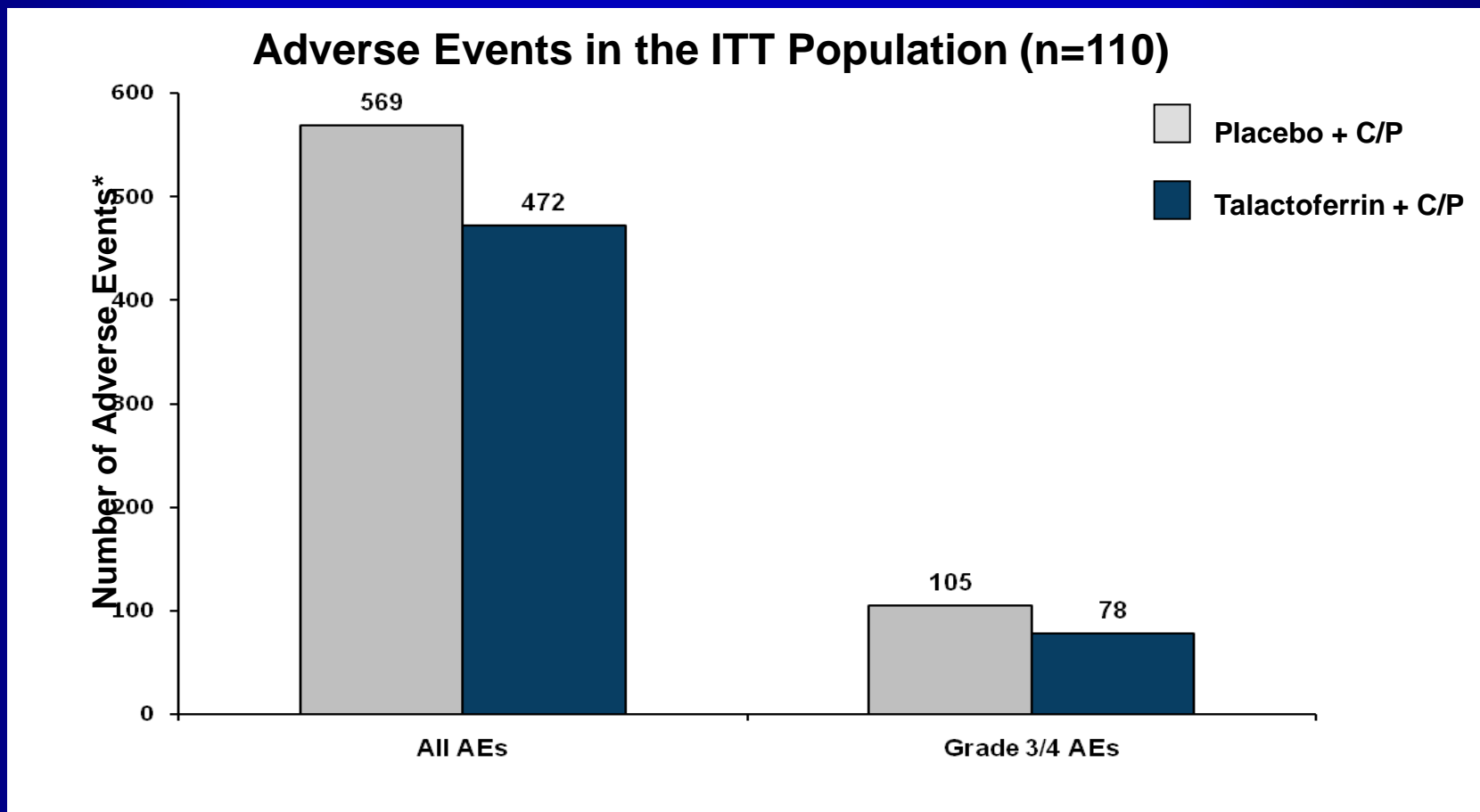
LF-0206 Secondary Endpoints: Encouraging Trends in OS, PFS, Duration of Response Despite TLF Treatment Time Limited to 18 weeks (per Protocol)

<u>Median values</u> <u>(in months)</u>	<u>PFS</u>		<u>OS</u>		<u>Duration of Response</u>
	ITT	Evaluable*	ITT	Evaluable*	Patients with confirmed response**
Placebo + C/P	4.2	4.2	8.5	8.5	5.4
TLF +C/P	7.0	7.0	10.4	11.3	7.5
Absolute Improvement	2.8	2.8	1.9	2.8	2.1
Relative Improvement	67%	67%	22%	33%	39%
Hazard Ratio	0.85	0.78	0.87	0.75	0.53

* Prospectively defined as patients who received at least one dose of Study Drug as well as one dose of C/P, and who had at least one CT scan after start of treatment
 • excludes patients who died (5), or dropped out (5) prior to first post-treatment CT)

** Duration of response (DOR) for patients with a confirmed response. N=38, with 23 and 15 patients in the TLF and Placebo arms, respectively. DOR is measured from the date of first occurrence of a confirmed response to the date of tumor progression or death. Patients who had not progressed (18) or were lost to follow-up (10) were censored to the date of the last known non-progression CT scan, as for the PFS analysis.

LF-0206: Adverse Events



Improvement

17%

26%

P-value**

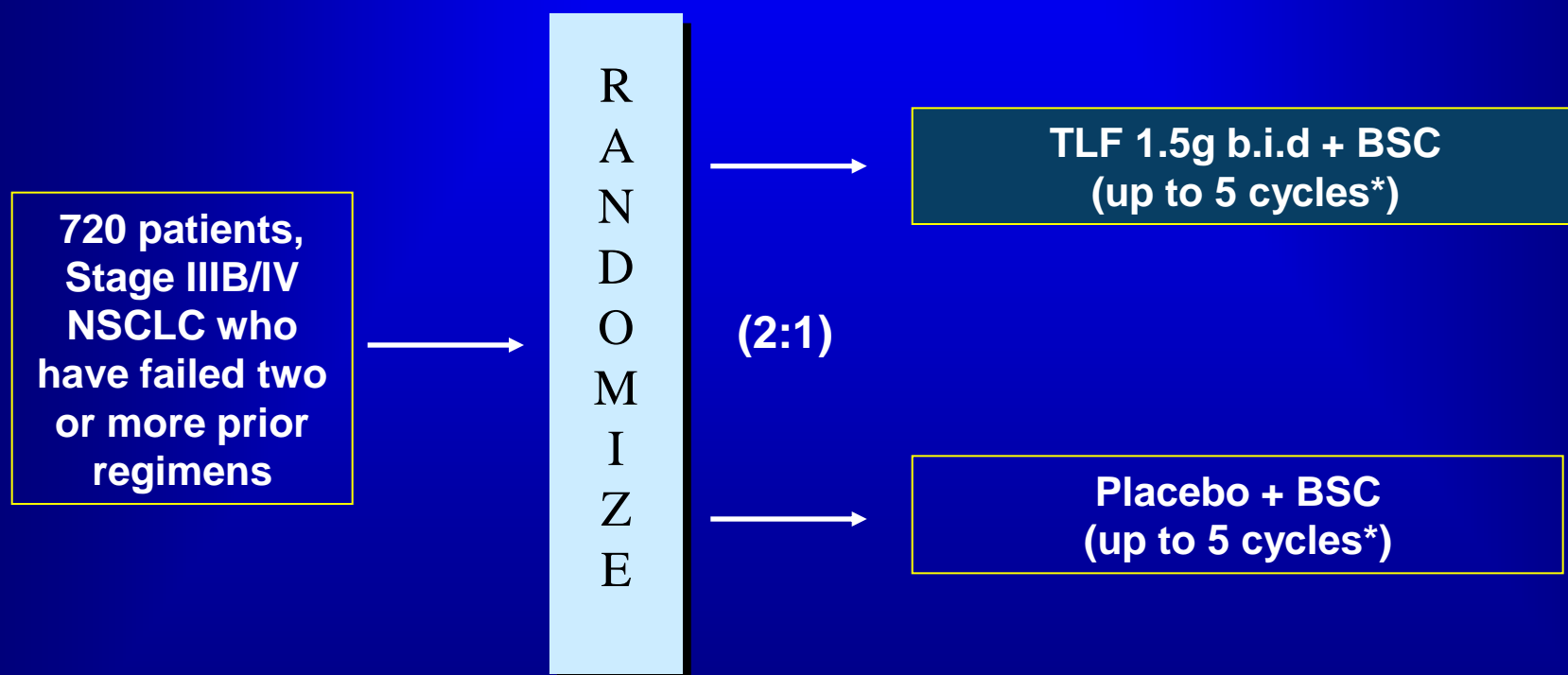
0.003

0.05

* AE reductions were consistent with the biological effects of talactoferrin.

** Two-tailed p-value obtained using Two-Proportion Binomial Test

FORTIS-M: A randomized, double-blind, placebo-controlled study of oral talactoferrin in addition to best supportive care in patients with NSCLC who have failed two or more prior regimens (LF-0207)



Stratifications: prior regimens; ECOG PS; geographical region

*One cycle = 12 weeks on, 2 weeks off

Future Directions of DC Therapy in NSCLC

- Employ DCs in early stage disease or in patients with minimal tumor burden
- Combination with other immunotherapy with different mechanisms of action to avoid immunotolerance
- Combination with targeted therapy or chemotherapy

The End