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Center of Experimental Medicine and Research (CeRMS) University of Turin
Janus Kinase (JAK) family of tyrosine kinases

Family members
- JAK1
- JAK2
- JAK3
- Tyk2

STATs: Signal Transducers and Activators of Transcription

Family members
- STAT1
- STAT2
- STAT3
- STAT4
- STAT5A/B
- STAT6
Canonical STAT3 signaling
Non Canonical STAT3 signaling

STAT3: A multifaceted oncogene

David E. Levy* and Giorgio Inghirami**

*Departments of Pathology and Microbiology and NYU Cancer Institute, New York University School of Medicine, New York, NY 10016; and **Department of Biomedical Sciences and Human Oncology, University of Turin and Center for Experimental Research and Medical Studies, 10060 Turin, Italy

Signal transducers and activators of transcription (STAT) proteins are a family of transcription factors first characterized for their role in cytokine signaling. These versatile regulators integrate extracellular signals and modulate gene expression, thereby controlling cell proliferation, survival, and differentiation. STAT3 is a key player in cancer, autoimmune diseases, and diabetes mellitus. It functions as a pro-oncogene in various malignancies and can be a target for therapy.

Stat3 regulates microtubules by antagonizing the depolymerization activity of stathmin

Dominic Chi Hiung Ng,1 Bao Hong Lin,1 Cheh Peng Lim,1 Guochang Huang,1 Tong Zhang,1 Valeria Poli,2 and Xinmin Cao1

1Signal Transduction Laboratory, Institute of Molecular and Cell Biology, Singapore 138673, Republic of Singapore
2Department of Genetics, Biology, and Biochemistry, University of Turin, 10126 Turin, Italy

Kasprzycka et al. (5) show that ALK+ tumor cell lines secrete the inhibitory cytokines IL-10 and TGF-β, express CD25 and FoxP3, and condition their growth medium to be immunosuppres-
The deregulated STAT3 activation is common event in human t

Table 1. STAT3 in the Context of Various Cancers: Validation as an Anticancer Target

<table>
<thead>
<tr>
<th>Cancers Characterized by Elevated STAT3 Expression or Activity</th>
<th>Poor Prognosis Linked to High STAT3 Levels</th>
<th>Upstream/Downstream Abnormalities of STAT3 Signaling</th>
<th>Xenograft Models Responsive to Inhibition of STAT3</th>
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<tbody>
<tr>
<td>Leukemia</td>
<td>Renal cell carcinoma</td>
<td>Elevated EGFR expression</td>
<td>Head and neck squamous cell carcinoma</td>
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<tr>
<td>Lymphomas</td>
<td>Colorectal cancer</td>
<td>Constitutively activated EGFR-RTK</td>
<td>Glioblastoma</td>
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<tr>
<td>Multiple myeloma</td>
<td>Ovarian carcinoma</td>
<td>Overexpression of SFKs</td>
<td>Myeloproliferative neoplasms</td>
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<td>Breast cancer</td>
<td>Gastric carcinoma</td>
<td>Hyperactivated JAKs</td>
<td>Renal cell carcinoma</td>
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<tr>
<td>Prostate carcinoma</td>
<td>Intestinal-type gastric adenocarcinoma</td>
<td>Elevated TGFα/IL-6</td>
<td>Breast cancer</td>
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<td>Lung cancer (non-small-cell)</td>
<td>Cervical squamous-cell carcinoma</td>
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<td>Lung adenocarcinoma</td>
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<td>Renal cell carcinoma lung cancer</td>
<td>Osteosarcoma</td>
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<td>Epithelial ovarian carcinoma</td>
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<td>Ovarian carcinoma</td>
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<td>Pancreatic adenocarcinoma</td>
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<td>Melanoma</td>
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<tr>
<td>Head and neck squamous cell carcinoma</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Johnston P.A. et al. Molecular Intervention 11, 18 (201
Peripheral T-cell lymphoma (PTCL) are rare cancers, which account for about 12% of all NHL worldwide. They are a heterogeneous group of neoplasms that display great variability in their clinical, morphological, immunophenotypic, cytogenetic and molecular features. The PTCL can be roughly subdivided into: specified and not otherwise specified (NOS). While

Overall incidence in USA ~5-6,000 cases a y
Overall Survival of patients with common Peripheral T-cell Lymphoma subtypes

(Modified from Armitage et al. JCO 2008)
Anaplastic Large Cell Lymphoma

The t(2;5)(p23;q35) translocation is associated with Ki-1 (CD30+) lymphoma


Fusion of a kinase gene, ALK, to a nuclear protein gene, NPM, in non-Hodgkin’s lymphoma

Morris S.W. et al. Science 1994;263(5151):1281-4
Timeline | Major events in the characterization of ALCL

**1988**
- Detection of the novel translocation t(2;5) associated with CD30^+ALCL

**1989**
- Identification of ALK in the t(2;5) translocation

**1994**
- Demonstration of ALK tumorigenicity in vivo

**1997**
- Detection of translocated ALK in other cancers

**1999**
- Generation of transgenic mice expressing NPM-ALK that develop lymphomas

**2000**
- Development of ALK inhibitors in vitro and in vivo

**2003**
- Proof of principle of 'ALK addiction' by specific short hairpin RNA

**2005**
- Clinical trial with anti-CD30 antibody in paediatric CD30^+ ALCL

**2006**
- New therapies for ALCL:
  - Combination of small molecules
  - Vaccination

**2007**
- ...

ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; NPM, nucleophosmin.

Chiarle et al., Nature Rev Cancer 2008
Translocations and fusion proteins involving the ALK gene in ALCL

Translocation  Frequency  Localization  
(NHL)

\( t(2;5) (\ p23 ;q35 ) \)  70-80% Cytoplasmic/Nuclear nucleolar

\( t(1;2) (\ q25 ;p23 ) \)  10-20% Cytoplasmic

\( t(2;3) (\ p23 ;q21 ) \)  2-5% Cytoplasmic

\( \text{inv}(2) (\ p23 ;q35 ) \)  2-5% Cytoplasmic

\( t(2;17) (\ p23 ;q23 ) \)  2-5% Cytoplasmic

\( t(2;19) (\ p23 ;q13,1 ) \)  - Cytoplasmic

\( t(2;2) (\ p23 ;q11-13 )? \) or \( \text{inv}(2) (\ p23 ;q11-13 )? \) - Nuclear membrane

\( t(X;2) (\ q11-12 ;p23 ) \) - Cell-Membrane

\( t(2;17) (\ p23 ;q25 ) \)  2-6% (NSCLC) Cytoplasmic

\( \text{EML4} \)  ALK
Chromosomal translocations involving anaplastic lymphoma kinase gene in cancers

<table>
<thead>
<tr>
<th>Disease</th>
<th>Fusion protein</th>
<th>Chromosomal abnormality</th>
<th>Principal references</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALCL</td>
<td>NPM–ALK</td>
<td>t(2;5)(p23;q35)</td>
<td>Morris et al. (1994) and Shiota et al. (1994)</td>
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<tr>
<td>ALCL</td>
<td>ALO17–ALK</td>
<td>t(2;17)(p23;q25)</td>
<td>Cools et al. (2002)</td>
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<td>ALCL</td>
<td>TFG–ALK</td>
<td>t(2;3)(p23;q21)</td>
<td>Hernández et al. (1999, 2002)</td>
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<tr>
<td>ALCL</td>
<td>MSN–ALK</td>
<td>t(2;X)(p32;q11–12)</td>
<td>Tort et al. (2001, 2004)</td>
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<tr>
<td>ALCL</td>
<td>TPM3–ALK</td>
<td>t(1;2)(q25;p23)</td>
<td>Lamant et al. (1999) and Siebert et al. (1999)</td>
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<tr>
<td>ALCL</td>
<td>TPM4–ALK</td>
<td>t(2;19)(p23;p13)</td>
<td>Meech et al. (2001)</td>
</tr>
<tr>
<td>ALCL</td>
<td>ATIC–ALK</td>
<td>inv(2)(p23;q35)</td>
<td>Colleoni et al. (2000), Ma et al. (2000), and Trinei et al. (2000)</td>
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<tr>
<td>ALCL</td>
<td>MYH9–ALK</td>
<td>t(2;22)(p23;q11–12)</td>
<td>Lamant et al. (2003)</td>
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<tr>
<td>ALCL</td>
<td>CLTC–ALK</td>
<td>t(2;17)(p23;q23)</td>
<td>Touriol et al. (2000)</td>
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<td>IMT</td>
<td>TPM3–ALK</td>
<td>t(1;2)(q25;p23)</td>
<td>Lawrence et al. (2000)</td>
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<td>IMT</td>
<td>TPM4–ALK</td>
<td>t(1;19)(p23;p13)</td>
<td>Lawrence et al. (2000)</td>
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<td>IMT</td>
<td>CLTC–ALK</td>
<td>t(2;17)(p23;q23)</td>
<td>Bridge et al. (2001) and Patel et al. (2007)</td>
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<tr>
<td>IMT</td>
<td>SEC31L1–ALK</td>
<td>t(2;4)(p23;q21)</td>
<td>Panagopoulos et al. (2006)</td>
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<tr>
<td>IMT</td>
<td>RANBP2–ALK</td>
<td>t(2;22)(p23;q13) inv(2)(p23;p15;q31)</td>
<td>Ma et al. (2003)</td>
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<tr>
<td>IMT</td>
<td>CARS–ALK</td>
<td>t(2;11)(2)(p23;p15;q31)</td>
<td>Cools et al. (2002) and Debelenko et al. (2003)</td>
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<td>NSCLC</td>
<td>EML4–ALK</td>
<td>inv(2)(p21;p23)</td>
<td>Rikova et al. (2007) and Soda et al. (2007)</td>
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<td>NSCLC</td>
<td>TFG–ALK</td>
<td>t(2;3)(p23;q21)</td>
<td>Rikova et al. (2007)</td>
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<tr>
<td>DLBCL</td>
<td>NPM–ALK</td>
<td>t(2;5)(p23;q35)</td>
<td>Adam et al. (2003) and Onciu et al. (2003)</td>
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<tr>
<td>DLBCL</td>
<td>CLTC–ALK</td>
<td>t(2;17)(p23;q23)</td>
<td>De Paepe et al. (2003)</td>
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<td>DLBCL</td>
<td>Unknown</td>
<td>ins(3'ALK)(4q22–24)</td>
<td>Stachurski et al. (2007)</td>
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<tr>
<td>DLBCL</td>
<td>SQSTM1–ALK</td>
<td>t(2;5)(p23–q3–3)</td>
<td>Takeuchi et al. (2010)</td>
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<td>SCC</td>
<td>TPM4–ALK</td>
<td>t(2;19)(p23;p13)</td>
<td>Du et al. (2007) and Jazii et al. (2006)</td>
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<tr>
<td>RCC</td>
<td>VCL–ALK</td>
<td>t(2;10)(p23;q22)</td>
<td>Debelenko et al. (2010)</td>
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</table>
Oncogenic signaling cascade activated by NPM-ALK
STAT3 silencing induces cell cycle arrest and apoptosis

Chiarle et al., 2008

STAT3 silencing induces cell cycle arrest and apoptosis

Piva et al. JCO, in press
ALK/STAT3 signature predicts ALK status in T-NHL patients

337 genes

shALK shSTAT3

ALCL 36
PTCL-NOS 28
AILT 6
T-Cells 20
Biological validation of new NPM-ALK putative targets

Inducible/drug specific NPM-ALK KO in ALCL cells and/or primary ALCL

Gene expression profile

Proteomic

All genome seq

DNA methylation

Up-regulated

Down-regulated

X number of candidates (A, B, C...Z)

Generation of 2 libraries

shRNA

cDNA

Transduction in ALCL cells with inducible NPM-ALK KD

NPM-ALK ON + A shRNA → Cell growth arrest - apoptosis

NPM-ALK KD + A cDNA → Partial rescue of the phenotype
ALK expression signature is largely dependent upon STAT3 activity.
The kinetics of STAT3-regulated genes

Platform: Illumina HumanHT-12 BeadChip (25,000 genes)
Differential Score: $p<0.001$, $\text{FC}>2$

DEG at least in one time-point 1730

Overlap with a previous STAT3 KD GEP experiment performed with 3 different shRNA at 84-96h
Identification and validation of STAT3 binding sites

Position Weight Matrix approach

<table>
<thead>
<tr>
<th>GENE</th>
<th>POSITION</th>
<th>SCORE</th>
<th>CONSERVATION</th>
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<tbody>
<tr>
<td>TNFRSF8</td>
<td>-3279</td>
<td>10.19</td>
<td>Bt</td>
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<tr>
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<td>-1257</td>
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<td>Cf</td>
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<td></td>
<td>-374</td>
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<td></td>
<td>19281</td>
<td>9.24</td>
<td>Bt</td>
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<td>GAS1</td>
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<td>1815</td>
<td>10.47</td>
<td>Mm</td>
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<td>12765</td>
<td>10.24</td>
<td>Cl, Bt</td>
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Validation of STAT3 binding sites by CHIP

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<th></th>
<th>Contr</th>
<th>+DOX 96h</th>
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<td>STAT3</td>
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<tr>
<td>IgG</td>
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<td>Input</td>
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<td>STAT3</td>
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<tr>
<td>Input</td>
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</table>

**GAPDH**

**SOCS3**

**IL2RA 3771**

**IL2RA 3589**

**IL2RA 28067**

**GAS1 209**

**GAS1 6227**

**GAS1 9870**
Mir17-92 overexpression rescues STAT3 KD of ALK+ ALCL cells

**TS-TTA + Doxi**

% Cell Death

Day 3  Day 6  Day 7  Day 8  Day 9


**JB6-TTA + DOXI**

% Cell Death

Day 3  Day 6  Day 7  Day 8  Day 9

JB6-TTA ctrl  JB6-TTA 17-92  JB6-TTA 17-92 Inv  JB6-TTA 17-92 II

**TS-TTA qRT-PCR: primer mir-19a**

$2^{ΔΔCt}$

**TS-TTA qRT-PCR: primer mir-92**

$2^{ΔΔCt}$
# Strategies and Challenges to Therapeutic Intervention into STAT3 Signaling

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Targets</th>
<th>Examples</th>
<th>Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibit phosphorylation/activation of STAT3</td>
<td>EGFR agonism, TKR activity, JAK activity, SFK activity</td>
<td>Cetuximab, panitumumab, Gefitinib, erlotinib, lapatinib, AG490, LS-104, ICNB1824, CEP-701, Dasatinib, AZD0530, bosutinib</td>
<td>Modest efficacy; development of resistance; myelosuppression, GI toxicity, and adverse events; kinase selectivity and cardiovascular toxicity</td>
</tr>
<tr>
<td>Inhibit intermolecular interactions that involve STAT3</td>
<td>STAT3 SH-2 domains</td>
<td>Oligopeptides designed from EGFR, gp130, and other receptor or pY-containing peptides; peptide epitamers; G-quartet oligonucleotides; small-molecule peptidomimetics</td>
<td>Poor cell permeability and efficacy; poor metabolic stability; poor selectivity for specific SH2 domains; potential for adverse events</td>
</tr>
<tr>
<td>Inhibit nuclear import/export of STAT3</td>
<td>Importins α3, α 5, α 7, Importin β, Exportin 1</td>
<td>Karyostatin 1A (effect on STAT3 undetermined), Leptomycin B and Raliladone A</td>
<td>Multicomponent nature of nuclear pore and translocation not fully determined; specificity for translocated proteins problematic</td>
</tr>
<tr>
<td>Inhibit STAT3-mediated transcription</td>
<td>DNA binding site of STAT3</td>
<td>dsODN decoys; peptide aptamers</td>
<td>Poor cell permeability without effective and specific delivery systems; poor metabolic stability</td>
</tr>
<tr>
<td>Natural products</td>
<td>Unspecified</td>
<td>Guggulsterone, honokiol, curcumin, resveratrol, flavopiridol, cucurbitacin</td>
<td>Specificity, potency, and efficacy, mechanism of action unknown</td>
</tr>
</tbody>
</table>

Drug discovery strategies

Mimicking peptides

Selected libraries
Compounds already In Clinics
Dr D. Frank
Dana Faber Institute

Large libraries
small molecules

Genomic libraries
shRNA
miRNA
How should we select the right models to test STAT3 inhibitors?

Cortese D.
PYRIMETHAMINE

STAT3 INHIBITORS

Supplementary Figure 1: (A) The effects of pyrimethamine on CD4.524 cells after 24 hours. (B) Western blot analysis showing the expression levels of p-STAT3, p-ALK, and ACTIN in TS, SU-DHL1, and CD4.524 cells treated with different concentrations of pyrimethamine. (C) The effect of pyrimethamine on apoptosis in TS, SU-DHL1, and CD4.524 cells after 48 hours.
LLL12

STAT3 INHIBITORS

MAC cell cycle

% phase arrest

CTRL 1μM 2.5μM 5μM

TS cell cycle

% phase arrest

CTRL 1μM 2.5μM 5μM

SU-DHL1 cell cycle

% phase arrest

CTRL 1μM 2.5μM 5μM

CEM cell cycle

% phase arrest

CTRL 1μM 2.5μM 5μM

TS | SU-DHL1

P-Y-100

p-ALK

p-STAT3

ALK

STAT3

CASPASE3

ACTIN

apoptosis

% cell death

CTRL 1μM 2.5μM 5μM

TS | SU-DHL1

MAC

CEM
From Human to Mouse and Back: “Tumorgraft” Models Surge in Popularity

By Ken Garber

Mouse xenograft models of cancer, understandably, have a terrible reputation. Although researchers and companies routinely use these human tumors in mice for preclinical drug testing, individual models poorly predict how drugs will act in the clinic. Retrospective reviews published by the National Cancer Institute in 2001 and the National Cancer Institute of Canada in 2003 came to the same conclusion: Drugs that work against cancer in xenograft mice rarely work in people with the same tumor, resistance to gemcitabine, the standard therapy for pancreatic cancer, and sensitivity to mitomycin C, a rarely used treatment. The patient—who first failed gemcitabine therapy—received mitomycin C and has been in remission for more than 2 years.

The trial’s principal investigator, Manuel Hidalgo, M.D., Ph.D., stressed that tumorgrafts, at present, can be of only limited use for individualizing patient treatment because of the time and resources necessary to create tumorgraft banks. Many
Where are we going with novel personalized cancer strategies?
ALCL primary and correspondent ALCL tumorgraft display identical immunoprofiles

Primary ALCL-1

Tumorgraft ALCL-1-T3

CD30

ALK

Perforin

CD30

ALK

Perforin
Preclinical therapeutic strategies

Fourth line resistant ALK+ ALCL

Treatment regimen

Placebo

Doxocubicin 10mg/kg/SD/iv

CEP28122 100mg/kg bid/os

Doxocubicin 10mg/kg/SD/iv

+ CEP28122

100mg/kg bid/os
Anti-ALK CEP28122 cures ALK+ ALCL tumorgraft
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Antonino Neri
Luca Agnelli

MBC
Flavio Cristofani
Guido Forni
Ferdinando di Cunto
Paolo Provero
Silvio Aime
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Fiorella Altruda

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IRCC-Candiolo
Enzo Medico

New York University
David E. Levy
Herman Yee

ISIS
James G Karras

Cell Signalig Technology
Robert Polakiewycz
Michael Comb

Cephalon Inc.
Bruce A. Ruggeri
Mangeng Cheng

The European T-cell Lymphoma Study Group

Politecnico Torino
Francesco Abate
Andrea Acquaviva
Elisa Ficcara

Columbia University
Raul Rabdan

RIC

REGIONE PIEMONTE

Con la ricerca, contro il cancro.

Berlucchi