# Early career grants

Eva Hellström-Lindberg

Chair doctoral studies
Dept of medicine

# Finding the right grant

- Identify the correct funding agency for your application
- Is there a specific call that is suitable for your application?
- What is the time frame, start date and duration that you need?
- Ask for advice

Local (KI, SLL)

Societies / funds

Governmental

International

Eva Hellström Lindberg, 2011

# Types of Grants

- Salary - post doc - 100% - 50%
  - For local positions (KI, SLL, societies)
  - For positions abroad (as above + EU, universities, local societies)
- Project grants
  - Dependent
  - Independent (small, or usually not until after post doc)
- Part of supervisor´s grants

# Before You Start

- What is your specific hypothesis?
- Could this proposal be defined as FINER?
  - Feasible, Interesting and Important, Novel, Ethical and Relevant?
- Is this a realistic project?
  - Do you have sufficient preliminary data?
  - Do you have the relevant competence, facilities, equipment, personnel, patient population, samples, time, motivation?
  - Are the appropriate ethical approvals in place?

# Initial Steps

- Do a thorough literature search
  - Is this done already?
- Clarify relation to Supervisor / Mentor
  - Is this your project or his/her's
- Get statistical advice!!!
- Consider collaboration, meet with collaborators and get appropriate letters of support
  - Be prepared to write these
- Meet to outline a research protocol
- Find out deadlines and what is required when

# Application

- Plan carefully
- Download and read paperwork carefully and check for deadlines –THESE ARE FINAL
- Test on-line application system
- Allow sufficient time for financial forms and Institutional sign off
- Remember that not all signatories may be there at the day of submission

# Writing

- Describe the basic hypothesis clearly and succinctly - let is rest and rewrite it!
- If the basic research question is not deemed important, everything else will fail
- Outline the research plan
  - What
  - How
  - Why
- Read instructions carefully and do not have own ideas about format

# Research Plan

- What exactly?
  - Overall aim
  - Specific aims and hypotheses
  - “To test these hypotheses I will address the following specific aims: .....
- Why?
  - Background and significance
  - Give credit to others - care literature review
  - Tell a story
- How?
  - Research design and methods
  - Biostatistical input to address power of study, sample size needs, animal models etc.

# How?

- Format varies in terms of space and detail required
- Go through the design of each aim in turn
- Make it logical
- Tell them what you think the pitfalls are and how you will address this
  - "It is possible the we will not demonstrate xxx. If so, we shall .....
- This is of particular importance if specific aim 2 is totally dependent upon a positive result from specific aim 1.
- Include why *you* are suitable to pursue this project
- Include why *your place* is best / sufficiently qualified to do this.
- "Sexy technologies" are not sexy if they are not relevant

# Advice

- Write it far enough in advance that you can have good feedback from you group and from other mentors
- Read your own text with critical eyes
- Be realistic
  - Do not be over-ambitious.
  - The study has to be feasible and achievable in the time frame and with resources/budget available
- Plan budget carefully and justify expenses well
- Do not exceed allowable page limits
- Limit appendices to essential documents
- Use easy to read type and include diagrams and figures where possible

# The Reviewers' Perspective

- Reviewers have had to read hundreds of grants over a limited period
- They may be intolerant but are usually careful
- When they see a sloppy grant they think "sloppy scientist"
- You may know you are smart, but so is everyone else who is applying!
- The trick is to make yourself look smarter than all the other applicants
- You can put yourself in the best light by asking the right question!

# If you had to read 100 grants, which one would you rather read?

Karolinska Institutet

The specific aims of Project 4 are tocharacterize and repair defects in T cells in patients with CLL and in the E-TCL1 transgenic mouse model of CLL. To identify the targets of T cell mediated immune responses against CLL cells of allogeneic and autologous T cells and to identify the targets of T cell mediated responses against CLL cells after allogeneic stem cell transplantation.

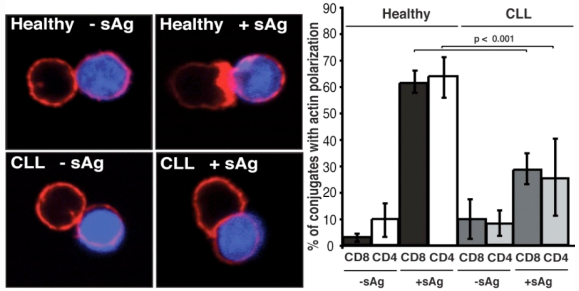
In specific aim 1 We hypothesize that T cells from CLL cancer patients become dysfunctional with tumor development and in previous work in this project had characterized the T cell defects in tumor-bearing patients by analyzing the global gene expression of highly purified $CD4^+$ and $CD8^+$ T cells from peripheral blood from individuals with CLL compared with age-matched healthy donors. Analysis revealed differentially expressed genes, mainly involved in cell differentiation and cytoskeletal formation pathways in $CD4^+$ T cells, and in cytoskeletal formation, vesicle trafficking, and cytotoxicity pathways in $CD8^+$ T cells. As complex cytoskeleton-dependent cellular processes are known to regulate T cell activation, we speculated that T cells from CLL patients would be defective in immunological function.
T cell antigen receptor (TCR) engagement and recognition of antigen induces dramatic morphological changes in T cells, characterized by polarization of the actin cytoskeleton and accumulation of F-actin at the site of contact with the APC, termed the immunological or immune synapse. This cellular signaling structure orchestrates the complex communication between the T cell and the APC in a way that ensures detailed antigen recognition and effective T cell responses. As part of this process, key receptors and signaling molecules are recruited to supramolecular activation clusters (SMACs); major components of the immune synapse. The central SMAC (c-SMAC) contains proteins including TCR, CD3 and Lck that co-cluster in the centre of the mature synapse site. A second zone surrounds the c-SMAC, the peripheral SMAC (p-SMAC), which on T cells is characterized by high concentrations of integrin leukocyte function-associated antigen (LFA-1, CD11a/CD18; $\alpha L\beta 2$ ). The LFA-1 ligand, intracellular adhesion molecule 1 (ICAM-1, CD54), is expressed in the p-SMAC of APCs. The p-SMAC is thought to provide adhesive anchoring of the T cell to the APC, while the c-SMAC forms a protected zone for TCR signaling. We hypothesized that T cells from cancer patients may inappropriately respond to APCs due to an inability to regulate actin remodeling effectively. We showed, by using both primary cells from CLL patients and using the transgenic mouse model of CLL ($E\mu$-TCL-1) that $CD4^+$ and $CD8^+$ T cells from tumor-bearing patients have an impaired ability to form immunological synapses. Our results show that critical immunological synapse formation steps are inhibited including conjugation of T cells with APCs, the subsequent polarization of F-actin, and the recruitment of TCRs, adhesion molecules, and actin cytoskeleton proteins to the synapse contact site. Moreover, we provided evidence that this immunological defect is induced in healthy allogeneic T lymphocytes by direct contact with tumor B cells, identifying a cellular mechanism whereby CLL tumor cells may inhibit immunological recognition facilitating disease progression.
We also identified the potential of improving this immune dysfunction in CLL using an immunomodulatory drug lenalidomide that appears capable of repairing F-actin polymerization and signaling at the immunological synapse. Lenalidomide, is a thalidomide analog, that is clinically active in patients with relapsed or refractory CLL, multiple myeloma and myelodysplastic syndrome. Lenalidomide has immune-activating properties with T cells including NKT cells. Thus, we examined the ability of lenalidomide to modulate formation of the immunological synapse in the autologous CLL patient setting. Pre-treatment of ex vivo $CD8^+$ or $CD4^+$ T cells and autologous CLL B cells with lenalidomide (24 h) before conjugation assays significantly ($p<0.01$) increased the number of conjugates showing F-actin polymerization at the immune synapse compared to untreated CLL cells ($p<0.01$) as well as the percentage of T cell conjugates formed . Treatment of both CLL B cells and CLL T cells with lenalidomide was required since treatment of either cell alone did not result in increased actin polymerization or recruitment of tyrosine phosphorylated protein to the synapse.

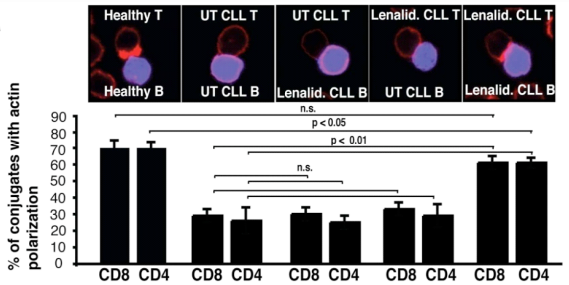
The specific aims of this project 4 are:

1. **To characterize and repair defects in T cells**
   a. **in patients with CLL**
   b. **in the E-TCL1 transgenic mouse model of CLL**
2. **To identify the targets of T cell mediated immune responses against CLL cells of allogeneic and autologous T cells**
3. **To identify the targets of T cell mediated responses against CLL cells after allogeneic stem cell transplantation.**

**In specific aim 1**

Based upon our previous work demonstrating the impact of CLL cells on host T cells and the demonstration that CLL cells could induce similar changes in allogeneic T cells from healthy donors, we have continued to attempt to identify the molecular mechanisms for this. We hypothesized that T cells from CLL cancer patients become dysfunctional with tumor development and in previous work in this project had characterized the T cell defects in tumor-bearing patients by analyzing the global gene expression of highly purified $CD4^+$ and $CD8^+$ T cells from peripheral blood from individuals with CLL compared with age-matched healthy donors. Analysis revealed differentially expressed genes, mainly involved in cell differentiation and cytoskeletal formation pathways in $CD4^+$ T cells, and in cytoskeletal formation, vesicle trafficking, and cytotoxicity pathways in $CD8^+$ T cells. As complex cytoskeleton-dependent cellular processes are known to regulate T cell activation, we speculated that T cells from CLL patients would be defective in immunological function and respond inappropriately to antigen presenting cells (APCs) due to an inability to regulate actin remodeling effectively. We have shown in both primary cells from CLL patients and in a transgenic mouse model of CLL ($E\mu$-TCL-1) that $CD4^+$ and $CD8^+$ T cells from tumor-bearing patients have an impaired ability to form immunological synapses (IS). Our results show that critical IS formation steps are inhibited including conjugation of T cells with APCs, the subsequent polarization of F-actin, and the recruitment of TCRs, adhesion molecules, and actin cytoskeleton proteins to the IS. Moreover, we have provided evidence that this immunological defect is induced in healthy allogeneic T lymphocytes by direct contact with CLL cells, identifying a cellular mechanism whereby CLL tumor cells may inhibit immunological recognition facilitating disease progression. We also identified the potential of improving this immune dysfunction in CLL using an immunomodulatory drug lenalidomide that appears capable of repairing F-actin polymerization and signaling at the immunological synapse. Lenalidomide is clinically active in patients with relapsed or refractory CLL, multiple myeloma and myelodysplastic syndrome. Lenalidomide has immune-activating properties with T cells including NKT cells. Therefore, we examined the ability of lenalidomide to modulate formation of the immunological synapse in the autologous



## Grants are usually read in the following order:

- Scientific summary
- Scientific summary
- Summary for the laymen
- Aims, objectives, hypotheses
- Research plan
- Significance
- Background
- Preliminary results

Karolinska Institutet

# Which part do you usually write in the last moment?

# Scientific summary

Karolinska Institutet

Background Background Background Background Background Background
Background Background Background Background Background Background
Background Background Background Background Background Background
Aims Objectives Hypothesis Aims Objectives Hypothesis Aims
Hypothesis Aims Objectives Hypothesis Aims Objectives Hypothesis
Research plan / Methods Research plan / Methods Research plan
Research plan / Methods Research plan / Methods Research plan
Research plan / Methods Research plan / Methods Research plan
Research plan / Methods Research plan / Methods Research plan
Research plan / Methods Research plan / Methods Research plan
Preliminary results Preliminary results Preliminary results Preliminary
Preliminary results Preliminary results Preliminary results Preliminary
Preliminary results Preliminary results Preliminary results Preliminary
Significance Significance Significance Significance Significance
Significance Significance Significance Significance Significance

Eva Hellström Lindberg, 2011

# Scientific summary

Karolinska Institutet

Background Background Background Background Background Background
Background Background Background Background Background Background
Preliminary results Preliminary results Preliminary results Preliminary
Background Background Background Background Background Background
Background Background Background Background Background Background
Preliminary results Preliminary results Preliminary results Preliminary
Background Background Background Background Background Background
Background Background Background Background Background Background
Background Background Background Background Background Background
Preliminary results Preliminary results Preliminary results Preliminary
Background Background Background Background Background Background
Research plan / Methods Research plan / Methods Research plan
Research plan / Methods Research plan / Methods Research plan
Significance Significance Significance Significance Significance