*E. coli* derived nanobodies for p53 detection in saliva
**Oral squamous cell carcinoma**

- OSCC – malignant tumor with 640,000 new cases annually worldwide
- Saliva testing can detect potential biomarkers for OSCC
- Elevated level of p53 protein was identified in OSCC patients at different stages of disease
Conventional & Next Generation Abs

- ~35 monoclonal (mAbs) are approved for therapeutic applications by European Medicines Agency & FDA

- Most Abs – chimeric/humanized

- Next-generation Ab fragments: antigen-binding fragments (Fab-s), single chain variable fragments (scFv-s), and heavy-chain Ab-s (HcAb-s)
HcAbs are found in sera of camelids

- No constant domain CH1, only variable domains: VhH-s or nanobodies

- **Nanobodies** (Nb) – smallest naturally occurring fragments (around 120 aa)
Nanobodies in Camelids
Detailed VH and VHH composition
Advantages of Nbs

- Small and soluble
- Highly stable
- Bind to target with high specificity
- Can be conjugated to other proteins without loss of function
- Can be expressed and secreted in many organisms including *E. coli*
Type I Secretion System
Dimerization of single chain VhH p53 nanobody
Hypothesis

Modified E. Coli having functional type I secretion system will be able to secrete VhHp53 nanobody that contains HlyA secretion signal

Specific aim

To purify VhHp53 nanobody secreted by E. coli in order to check its affinity for p53 protein

Significance

E. Coli derived hcAbs for VhHp53 will help to reduce the time spent on diagnostics of OSCC & to lower the costs
Methodology

1. Transforming *E. coli* with hly*B* hly*D* genes in pVDL9.3 (Cm) with pUC57 vector encoding Vhhp53 genes
2. Induce expression of the protein with 1uM IPTG (6, 8 and 10 hours)
3. Measure photometric intensity (Varioscan flash 4.00.53)
4. Purify proteins from the media with Ni/NTA columns
5. SDS PAGE
6. Measure concentration (Nanodrop A280)
7. Check the affinity of obtained nanobodies to p53 protein
Photometric intensity of Protein expression in *E. coli*

![Graph showing photometric intensity of Protein expression in E. coli.](image-url)
SDS PAGE of purified Nb
Concentration of VhHp53 protein after purification with Ni/NTA column

1\textsuperscript{st} elution: 46.95 ng/ul

2\textsuperscript{nd} elution: 123.16 ng/ul
Conclusion/Prospective work

- Obtained pure VhH p53
- Participated in Interlab Study

Next step: to test the affinity of this nanobody for cancer biomarker p53

Future plan: to use this protein-nanobody interaction to design the biosensor for the detection of p53 in saliva samples for OSCC diagnosis
Acknowledgements

- School of Science and Technology, Nazarbayev University
- Technopark, Nazarbayev University
- Damira Kanayeva, PhD, Nazarbayev University, Astana, Kazakhstan
- Luiz Angel, PhD, CSIC, Madrid, Spain
- Rebecca Bocanegra, CSIC, Madrid, Spain
- Ana Cuervo, CSIC, Madrid, Spain
- Kenneth Alibek, MD, PhD, Nazarbayev University, Astana, Kazakhstan
- Gonzalo Hortelano, PhD, Nazarbayev University
- Alexander Shustov, PhD, NCB, Astana, Kazakhstan
- Sholpan Kauanova, Nazarbayev University, Astana, Kazakhstan
- Nurgul Imangali, Nazarbayev University, Astana, Kazakhstan
- Madina Shaimerdenova, Nazarbayev University, Astana, Kazakhstan
Our Team


Sircar, A. (2010). *Computational antibody structure prediction and antibody-antigen docking*. THE JOHNS HOPKINS UNIVERSITY.


References
Thank you very much for your attention!!!