

Screen for inhibitors of cell migration in cancer metastasis using adenoviral knock-down

Remko de Pril*, Annemarie Lekkerkerker*, Ester Frische*, Desiré van Steenhoven*, Ilhem Maghrani*, Tim Perera#, Janine Arts#, Martin Page#, David Fischer* and Richard Janssen*
 * Galapagos, P.O.Box 127, 2300 AC Leiden, the Netherlands
 # Ortho Biotech Oncology R&D, Turnhoutseweg 30, B-2340 Beerse, Belgium
 E-mail: remko.depril@glpg.com

Metastasis

Enhanced cell migration is a hallmark of metastatic cancer cells. The propensity of cancer cells to close an open wound in a cell monolayer is thought to predict this ability. We set-up a target discovery and validation program for cell migration in cancer metastasis using our adenoviral knock-down library.

Approach

Galapagos' target discovery platform is based on screening proprietary adenoviral knock-down libraries ("SilenceSelect®") targeting the drugable human genome. This library currently contains over 12,000 human shRNA vectors with a redundancy of 3 shRNAs per transcript. The combination of the adenoviral knock-down technology coupled with screening in human cells is designed to identify critical proteins in disease pathways.

Wound healing assay

To establish a wound healing assay, we used a human PC-3 prostate adenocarcinoma cell line. A 96-pin scratch tool was designed to apply a constant mechanical scratch-wound in a cellular monolayer. After scratch formation plates were washed and refreshed on a liquid handling robot. Cells were fixed at a preset time period after scratching and the plates were imaged on an InCell Analyzer1000.

Invasion assay

To validate the inhibitory effect of the knock-down constructs in a secondary biological assay we developed a 3D invasion assay. We used human A549 lung carcinoma cells which were transferred to matrigel coated Boyden chambers. We used FCS and EGF as chemoattractant and after invasion cells were stained with Phalloidin and imaged on a BD Pathway 435.

Image analysis

For the wound healing, an algorithm was made in-house with InCell Developer software based on transmitted light images. The algorithm is based on the measurement of open space, which comprises the area of the wound and as such identifies genes whose knock-down inhibit cell migration.

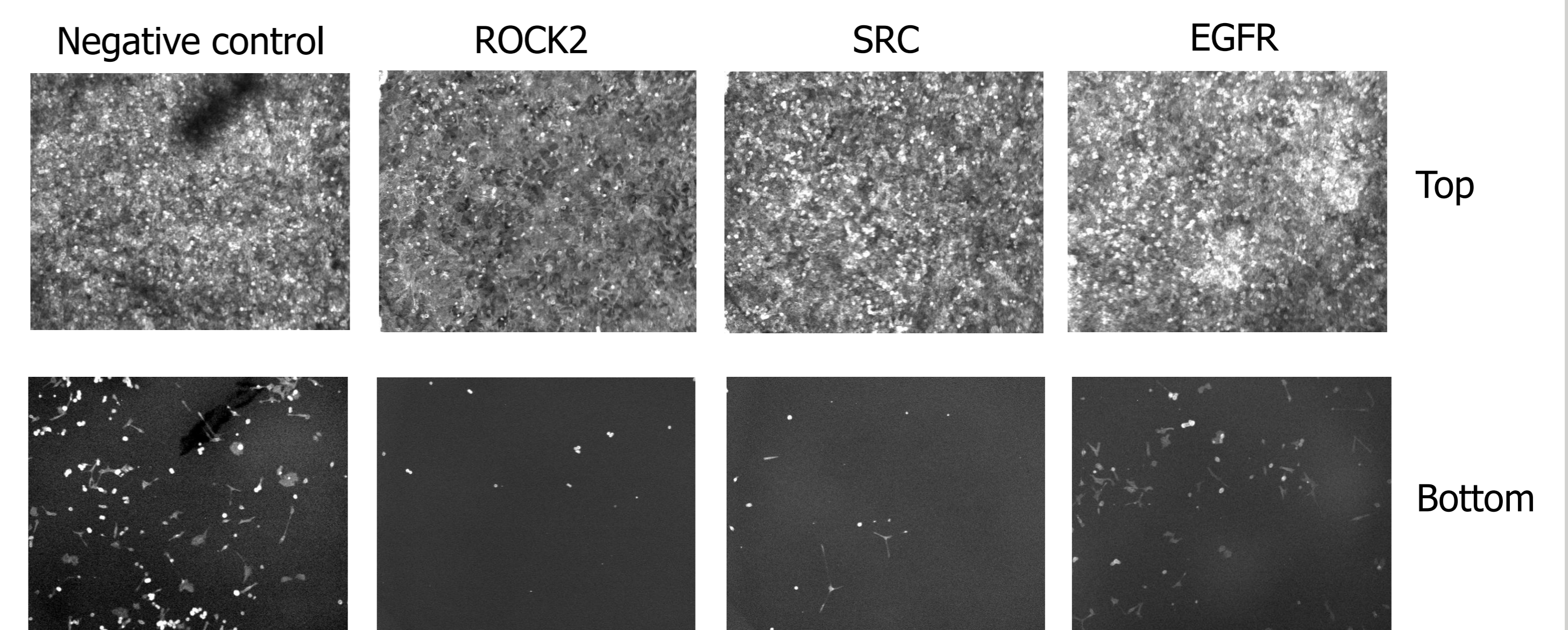
For the secondary assay, cells were segmented using Phalloidin images to measure the number of cells that invaded. The algorithm was developed in-house using BD Attovision software.

Validation of motility hits in 3D invasion

We have successfully completed the primary screen and rescreen of our shRNA library in the wound healing assay. The targets that inhibit motility are currently validated for their effect in 3D invasion using Boyden chambers.

A549 cells are transduced with the targets from the primary screen and transferred to the top compartment. FCS and EGF were used as chemoattractant and cells were stained after 48 hours.

Therefore, we use confocal imaging on a Pathway 435 of both the seeded cells in the top compartment and the invaded cells on the bottom of the filter. Using this approach, we have identified a number of control genes which inhibit invasion through matrigel. Accordingly, we will test whether the hits that inhibit motility in 2D additionally affect cell invasion in 3D.

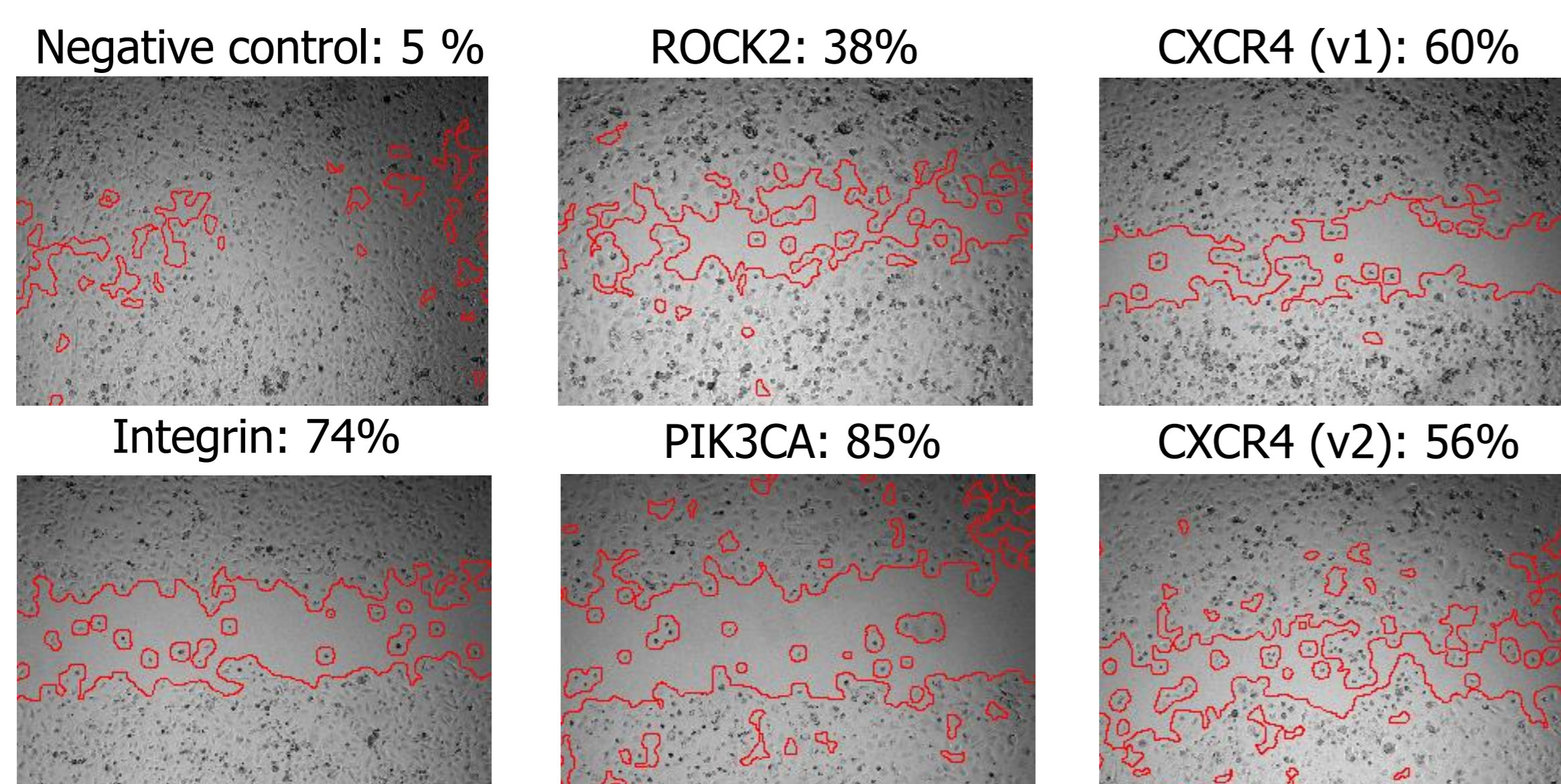


Inhibition of invasion using positive controls. Representative images from the top and bottom of the filter 48 hours after transfer to the Boyden chamber.

CXCR4 knock-down inhibits wound healing

For screening purposes, PC-3 cells seeded on collagen-IV coated plates were transduced with adenoviral knock-down constructs targeting different genes. The effect of different functional positive controls is shown.

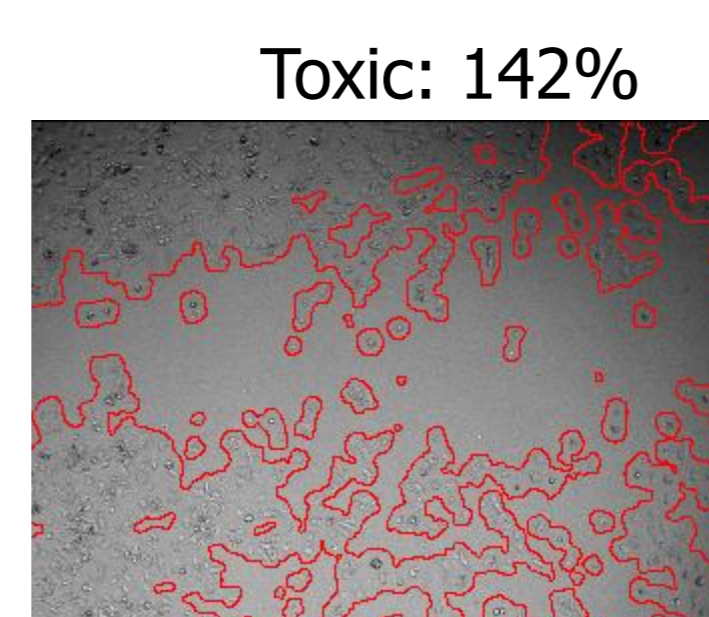
Two independent knock-down constructs targeting CXCR4, a known player in motility, demonstrated a clear reduction in motility of the PC-3 cells.



Inhibition of wound healing using positive controls. Representative images of controls at 8 hours after scratch formation. % open space is shown. Red overlay shows the open space segmentation.

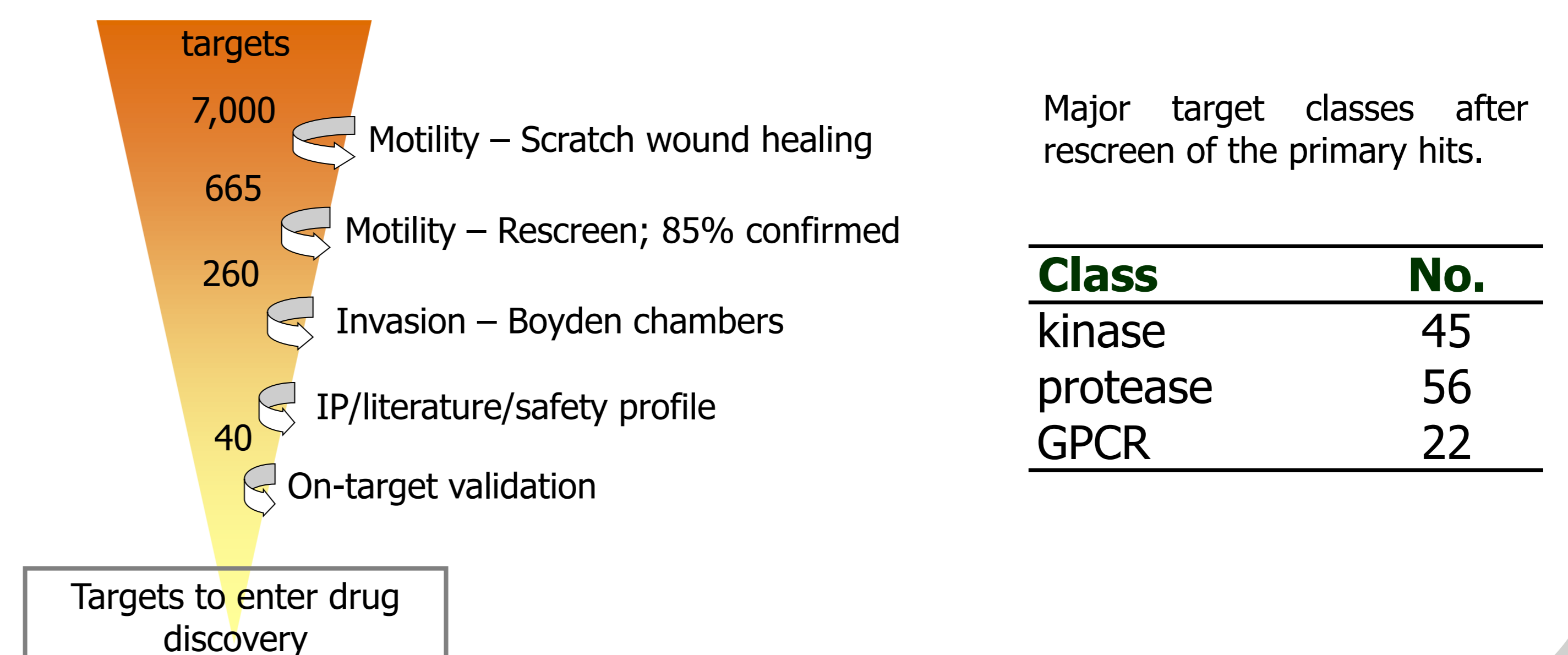
Counter-screen for toxicity

Genes that either affect proliferation, cell adherence, or viability can be counter screened as these disrupt the monolayer. These constructs result in an open area which exceeds the original scratch, as shown.



Target discovery and validation approach

Overview of adenoviral target discovery and consecutive attrition by secondary assays. Following the invasion, an on-target validation assay is performed to exclude off-target effects.



Conclusions

- ✓ Using our adenoviral shRNA knock-down library we have established a high-throughput wound healing assay
- ✓ Two knock-down constructs targeting a known player in motility, CXCR4, inhibit wound healing, validating our set-up
- ✓ We identified a high number of novel genes associated with tumor motility
- ✓ We have developed a 3D invasion assay that will be used for validation of the hits from our SilenceSelect screen.