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Introducing a new, long term screening methodology for identifying compounds that delay senescence onset

Website

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Introduction

Organismal aging causes decline in a wide range of cellular processes, resulting in diverse diseases and disabilities in the elderly, the treatment of which is potentially worth billons. Accumulation of senescent cells throughout tissues is causally associated with many chronic age-related diseases such as neurodegeneration, sarcopenia and inflammaging[1]. Thus, many companies are investigating compounds that either (a) aim to kill senescent cells (senolytics), which are being tested currently in clinical trials, or (b) compounds that reduce the damaging inflammatory secretion of senescent cells (senomorphics)[2]. However, the pathways targeted by senolytics are not unique to senescent cells, and this leads to potential off-target toxicity in healthy proliferative cells[2]. Instead, Five Alarm Bio's therapeutic strategy focusses on delaying the onset of senescence in cells through the development of novel small molecules. Here we introduce our new methodology for this purpose: The Limiting Dilution Assay.

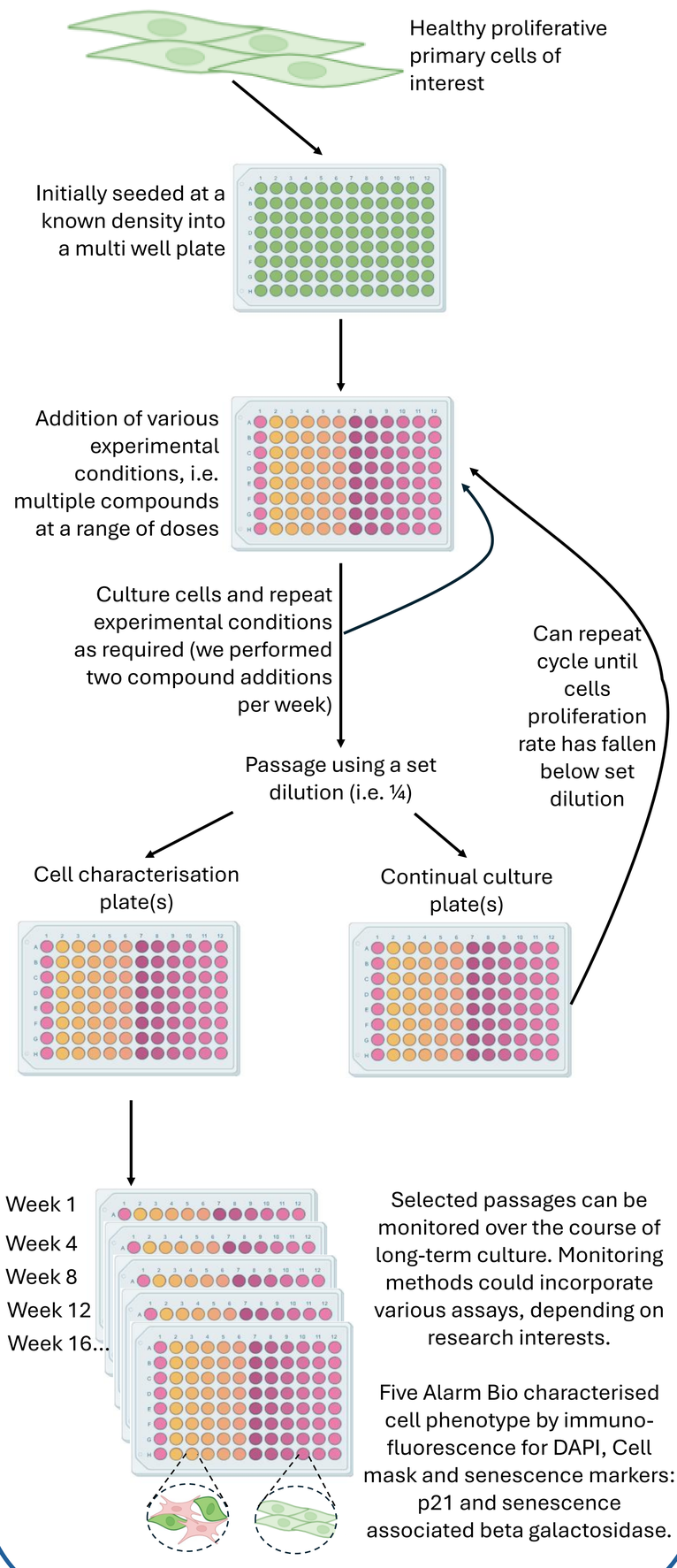
Who we are

Five Alarm Bio is developing a small molecule approach to boost the body's defence to ageing, based on a new understanding of how the chemical damage associated with age accumulates in cells. Five Alarm Bio plans to deploy our discoveries to develop safe and effective treatments for a range of age-related disease and disabilities. Founded in 2016 by three experienced entrepreneurs, Five Alarm Bio is based at Babraham Campus near Cambridge, UK, and is currently fundraising.

Limiting Dilution Assay

Method

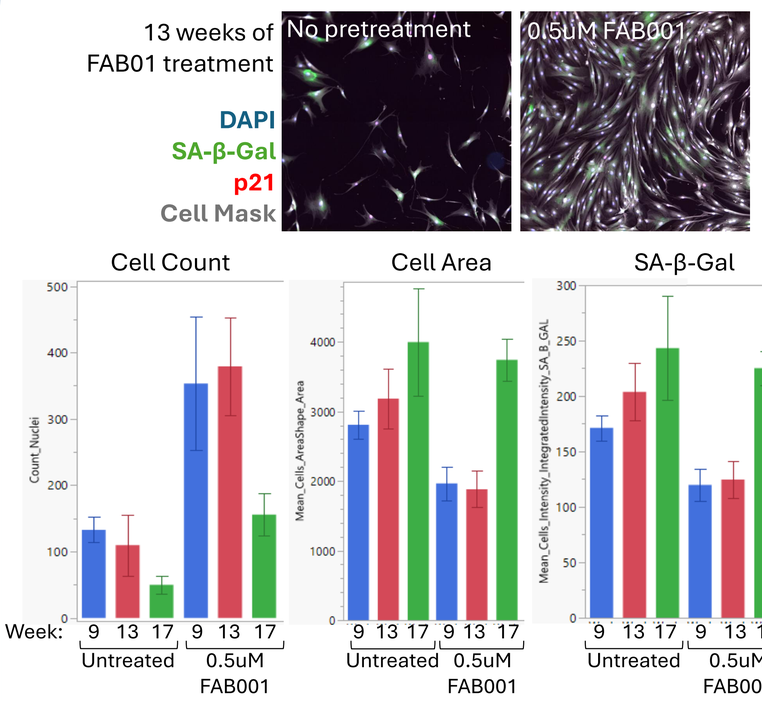
A new high throughput screening tool for assessing the effects of long-term treatment of primary cells with potential ageing-protective compounds. This innovative approach uses replicative senescence, which accounts for the accumulation of chemical damage, rather than stress-based techniques, modelling the process of the accumulation of senescent cells in the body, thus increasing clinical translatability.



Selected passages can be monitored over the course of long-term culture. Monitoring methods could incorporate various assays, depending on research interests.

Five Alarm Bio characterised cell phenotype by immuno-fluorescence for DAPI, Cell mask and senescence markers: p21 and senescence associated beta galactosidase.

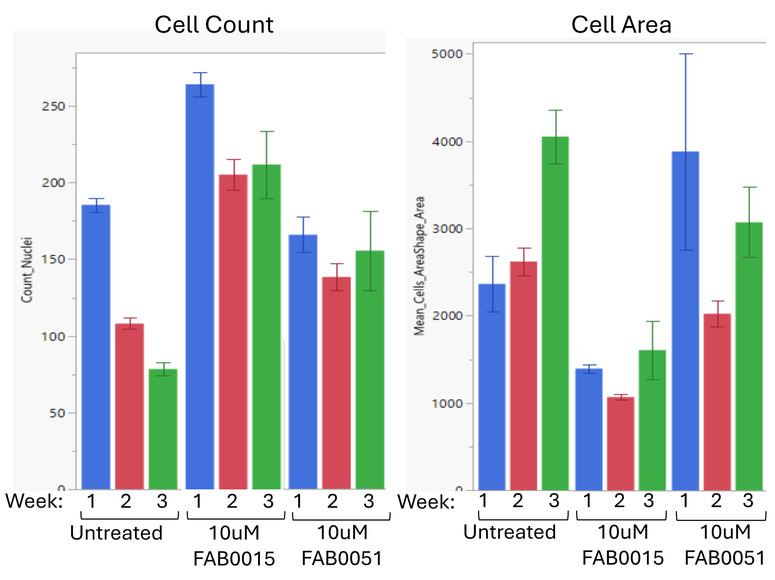
Example Results – Human Dermal Fibroblasts



FAB001 reduces senescence marker expression

Human dermal fibroblasts were treated with 0.5 μM FAB001 for up to 17 weeks then examined by immunofluorescence microscopy. Analysis revealed that treatment reduced the expression of senescence markers such as SA-β-Gal and p21. Morphological parameters associated with senescence such as increased cell area were also notably decreased.

Example Results – Human Myoblasts



FAB novel compounds delay the onset of both reduced proliferation and senescence linked morphological changes

Human Myoblasts were treated with 10 μM of novel FAB compounds FAB015 and FAB051 for 3 weeks then examined by immuno-fluorescence microscopy. Analysis revealed that treatment increased cell number and reduced cell area compared to untreated controls, most notably by FAB015.

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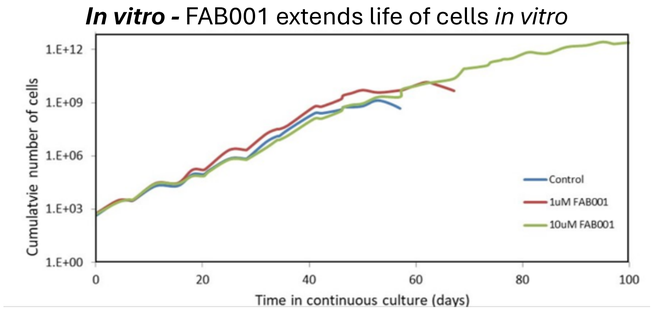
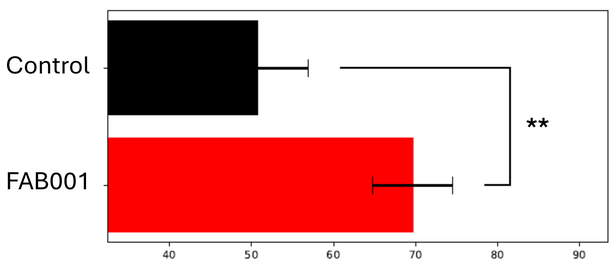
Initial Data

***In vitro* -** FAB001 extends life of cells *in vitro*



Probe compound FAB001 increases the maximum population doublings of primary human dermal fibroblasts

***In vivo* -** FAB001 extends healthy movement time

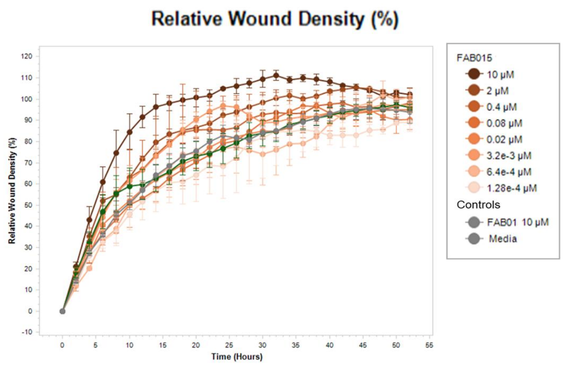


Caenorhabditis elegans (roundworms)

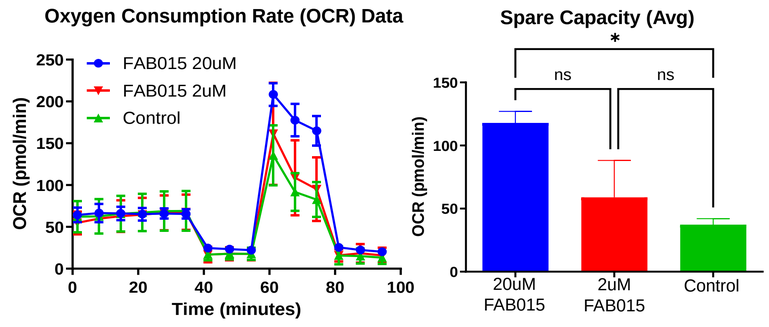
Probe compound FAB001 enhances *C. elegans* longevity

C. elegans were exposed either 5 mM FAB001 or vehicle and the fraction of worms moving determined by video microscopy and image analysis. This assays the healthy movement longevity of the worms. FAB001 significantly improves the healthy motility of *C. elegans*. Notably, FAB001 is not toxic to the worms at 5 mM.

Further Exploration of Novel Compounds Using Other *In Vitro* Assays



Novel FAB compound FAB015 accelerates rate of keratinocytes wound healing in an *in vitro* scratch assay.



Novel FAB compound FAB015 increases the spare respiratory capacity of human dermal fibroblasts, improving their ability to respond to stress.

Conclusion

Our early work showed that a small probe molecule improved longevity in a *C. elegans* model and reduces senescence marker expression in an *in vitro* replicative senescence assay. Subsequently, we developed a novel tool, the Limiting Dilution Assay, that allows higher-throughput exploration of experimental conditions that might delay senescence onset in a variety of primary human cells. This assay was incorporated by Five Alarm Bio into our pipeline after a high-content phenotypic compound screen which identified a subset of promising novel compounds. We have used the limiting dilution assay to explore the effects of novel compounds in different primary cells, with FAB015 being most promising. This compound was then tested in other *in vitro* assays, including an *in vitro* scratch wound model in human keratinocytes, human dermal fibroblasts and human myoblasts and measurements of mitochondrial oxygen composition rates. In each case FAB015 demonstrated positive results. We continue to develop and explore the effects of this and other of Five Alarm Bios novel small molecules.

Acknowledgments

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References: (1) Chaib, S. *et al.*, *Nat Med.*, (2022), Cellular senescence and senolytics: the path to the clinic **(2)** Lee. S. *et al.*, *Mech Ageing Dev.*, (2021) A guide to senolytic intervention in neurodegenerative disease