Development of a monitoring and treatment regimen for NSG mice displaying phenotypic tarsal joint swelling and hock lesions

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NSG (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ) mice are **severely immunocompromised** and require careful handling and strict biosafety protocols to maintain their health status.

Despite their genetic mutations, NSG mice typically do not display any gross physical or behavioural abnormalities (JAX, 2024). However, in recent years, we have observed **a subset of NSGs (~30%)** in our research labs developing a **progressive tarsal lesion phenotype** similar to that previously reported by MacLeod *et al.* (2017) in huNOG mice and Campagna *et al.* (2021) in NSG mice.

The phenotype initially presents as either unilateral or bilateral **rounding of the hock**, progressing to **swelling and reddening of the tarsal joint**, eventually advancing to **ulceration of the skin**. **Toe curl, grip strength and gait** are typically also impacted in the affected limb(s).

Using one of our internal R&D studies, we developed a scoring system and treatment plan to support affected animals through the acute phase, and subsequently collected samples to investigate the pathological changes occurring in the affected hocks.

Phenotype

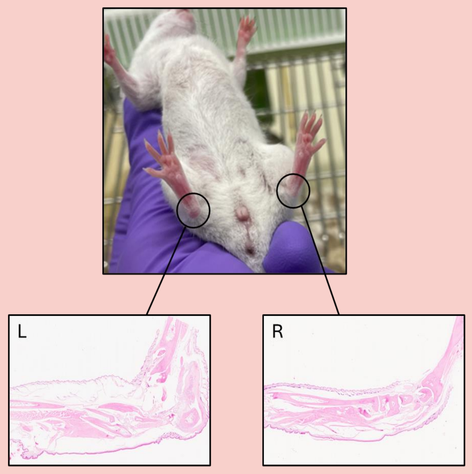

Figure 1. Comparison of an affected left hock and healthy right hock in a female NSG mouse. The left hock displays reddening and swelling of the heel *in vivo*. Histopathology indicated new cartilage and bone growth within the soft tissues of the hock. The right hock appears healthy *in vivo*, and histopathology was unremarkable.

Monitoring & Treatment

Case Study:

- 5 female NSGs from Charles River UK arrived on 04/12/23 aged 6-7 weeks, were housed in an IVC under standard conditions, and acclimatised for 10 days.
- Tumour cells were injected into the mammary fat pad on 14/12/2023. Tumour volume and body weights were measured 3x weekly for 8 weeks. Animals did not receive any anti-cancer agents during the study.
- The first animal developed hock issues on 08/01/24 (4th week of study). Hock issues were observed in 4/5 animals by study end.
- A treatment regimen was developed in collaboration with the University of Cambridge NACWO/NVS team: a drop of **Metacam (NSAID) orally twice daily for up to 10 days. Sudocrem applied to hock lesions once daily.**
- Tumour growth was not impacted by Metacam treatment. All animals completed the study with minimal welfare implications or impact to our study data.

Figure 2. Example of hock phenotype development and treatment. Hock lesion, reddening, and swelling of both tarsal joints with slight toe curl. The mouse also presented with flat-footed gait and intermittent weight bearing during the acute treatment phase, however grip strength was normal. Metacam was administered twice daily for 7 days. The hock lesions scabbed and eventually healed, however the morphological changes (swelling, pinkness, altered gait) remained until study completion.

Table 1. Our proposed clinical scoring system to monitor progression of NSG hock issues. During the acute phase, animals are treated with Metacam for up to 10 days, and daily scores taken to assess for positive treatment response. If animals present with hock lesions, sudocrem is also applied daily.

Clinical Score	0	1	2	3
Lesion	Normal	Skin thin and shiny	First skin layer broken	Wound through multiple tissue layers. Complications e.g. infection, necrosis
Swelling	No swelling	Rounding of hock	Widening of hock, darker shade of pink	Active inflammation, very red
Toe curling	Toes splayed	Toes together, minimal curling	Toes together, curled	Toes together, clenched
Grip	Normal	Strong grip	Weak grip	No grip
Movement	Normal	Slight change, not immediately obvious	Significant change e.g. waddling, rolling, or dragging foot	Loss of movement, hind limb paralysis

Husbandry & housing refinements:

- Bedding change from Aspen Eco Pure chips to **Alpha Dri**: less irritant and softer on the paws
- Animals **handled on a soft** surface only i.e. avoiding wire cage bars
- NSGs appear to be quite an **anxious strain**, so providing **additional enrichment** is beneficial:
  - Seeds scattered in bedding to encourage foraging behaviour
  - Nesting material changed/provided more frequently (avid nest builders)
  - Chew blocks given to provide extra stimulus/discourage tail nipping
- **Avoid the use of wheels or discs** in the cage

Pathology

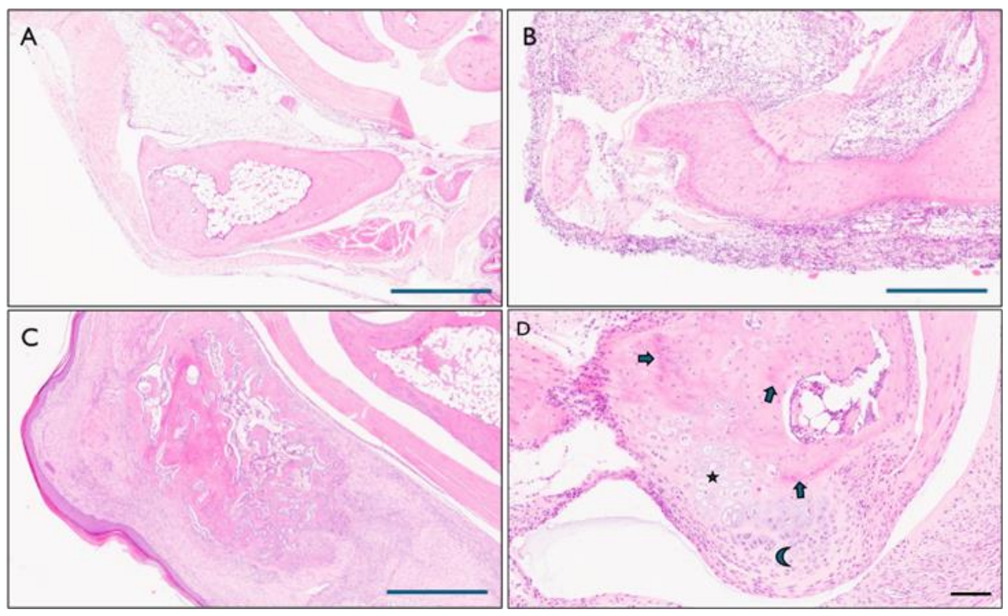

Figure 3. Low power microscopic images of hock regions with skin removed and calcaneum and underlying subcutaneous tissue visible. (A) unremarkable, healthy tissue. (B) Acute inflammation and partial hyperplastic changes in subcutaneous tissue. (C) Acute inflammation, hyperplastic changes in the skin, and proliferative and metaplastic changes in the cartilage. Marked changes in the bone structure. (H&E Stain, 5x, Scale bar = 500 µm). (D) Section of calcaneus showing denser areas of recent and immature bone formations (arrowheads), chondrocyte hypertrophy (star) and increased chondrocyte proliferation (crescent). Proliferative and hypertrophic chondrocytes are important steps in endochondral ossification – the replacement of cartilage with bone. (H&E stain, 10x, Scale bar = 100 µm).

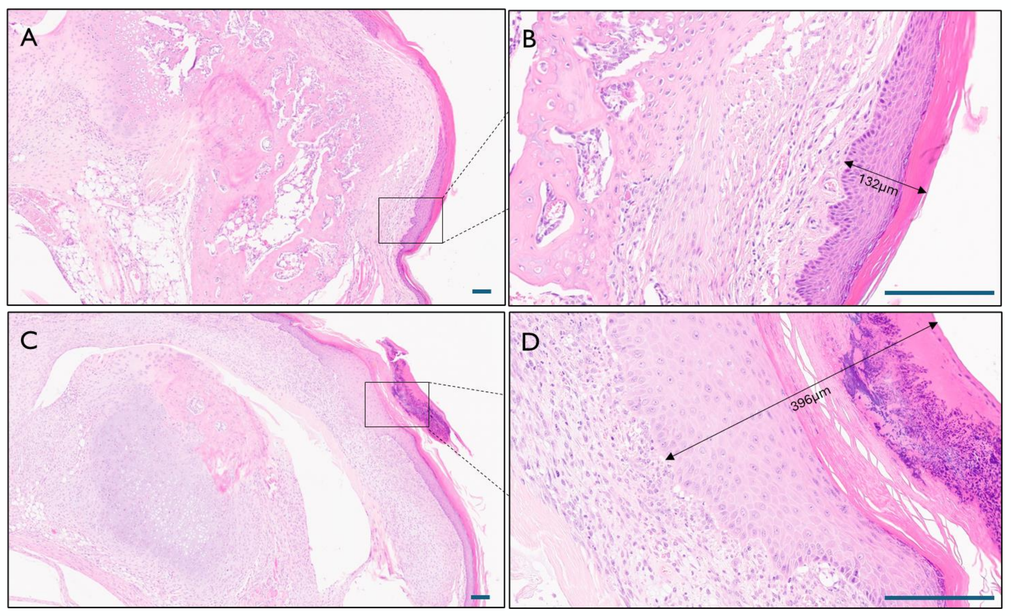

Figure 4. Structural comparisons of skin between (A+B) a mouse presenting with inflammation and new bone and cartilage growth, and (C+D) a mouse demonstrating inflammation alongside keratosis and scabbing. The epidermis and dermis is markedly thicker when inflamed. (H&E stain, A+C 4x, B+D 20x, Scale bar = 100 µm).

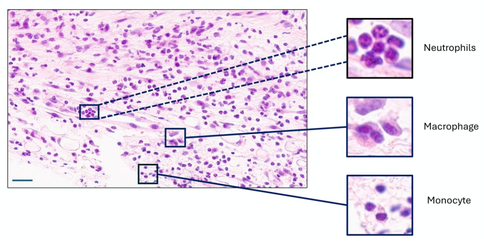

Figure 5. Subcutaneous tissue of mouse hind paw displaying acute inflammation. Monocytes and neutrophils are identifiable throughout alongside the presence of defective macrophage. (H&E stain, 40x, Scale bar = 25 µm).

Key Points

- There is acute inflammation and **non-reversible, ectopic formation of cartilage and bone** within the calcaneus tendon and heels.
- Our hypothesis is a **mass expansion of mesenchymal cells** is occurring in response to a trauma event (e.g. hyperextension or pressure on joint), leading to **endochondral ossification** (EO). In the EO bone formation pathway, mesenchymal tissue initially forms immature cartilage tissue which is then replaced with bone. The morphological changes observed *in vivo* e.g. swelling, lesions, change in gait and grip strength are a consequence of metaplastic cartilage and bone growth in the heel.
- Metacam provides **rapid pain relief** and **prevents further inflammation** occurring, thereby halting further lesion progression.
- The phenotype trigger is unknown. **Increased stress levels** appear to contribute to the development of these hock issues.

References

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