



Q & A

JNB = Julia-Newton Bishop

DW = David Whiteman

Q (anon): Were the subjects in your case studies wearing no sunscreen when outside?

JNB: we asked questions about sunscreen usage but only in general terms. The questionnaire we used was one developed by the Australian epidemiologists (Armstrong et al) as it had been evaluated and then used in many studies. Moreover, we were subsequently then able to compare responses using the same questionnaire in UK and Australian participants [1]. This required participants to answer questions about sun exposure and sunburn by decade through life which for most participants was a complex task. There were no specific questions about sunscreen use in this relative to each time period not least because it was felt that compliance would be very poor if we complicated the questions even more, and that the bias of recall would be extreme. Sunscreen usage is anyway very difficult to quantitate accurately as protection depends upon the nature of the sunscreen, how thickly it is applied and how often.

DW: in the experimental study in which subjects were exposed to 2.0 MED of solar-simulated radiation, we exposed the skin of the lower back/upper buttock, which is not habitually sun exposed.

Q. Marta Szell: We had some results (twin studies) some 10 years ago on the effect of neonatal blue light exposure (against jaundice) and clearly demonstrated increases in the number of nevi. Have you ever incorporated this factor into your epidemiology studies?

JNB: this was not a part of the Australian questionnaire and we did not ask this question as an addition. Although we recruited over 900 melanoma cases and 500 controls to the study the number of adults with an average in the 6th decade who were likely to have been exposed to blue light would likely have been too small to rely upon any data generated by such a question. Case control studies would have to be considerably larger (thousands of participants) to be confident in identifying risk associated with rare events.

DW: no – we have never asked about blue light in any of our studies. Their spectrum (380-550 nm) consists mainly of visible light with a peak at 450 nm and a minor component of UV light. The literature on this in relation to skin cancer and melanoma is small, and studies appear to have mostly null findings. As Julia notes, statistical power is a big problem with

these studies, as the exposure (blue light for hyperbilirubinaemia) is rare, as is the outcome (melanoma).

Bauer J, Büttner P, Luther H, Wiecker TS, Möhrle M, Garbe C. Blue light phototherapy of neonatal jaundice does not increase the risk for melanocytic nevus development. *Archives of dermatology*. 2004 Apr 1;140(4):493-4.

Berg P, Lindelöf B. Is Phototherapy in Neonates a Risk Factor for Malignant Melanoma Development?: A Preliminary Case-Control Study. *Archives of pediatrics & adolescent medicine*. 1997 Dec 1;151(12):1185-7.

Brewster DH, Tucker JS, Fleming M, Morris C, Stockton DL, Lloyd DJ, Bhattacharya S, Chalmers JW. Risk of skin cancer after neonatal phototherapy: retrospective cohort study. *Archives of disease in childhood*. 2010 Oct 1;95(10):826-31.

Q. Eli Sprecher: How much have we learned about non cell-related elements in melanoma pathogenesis from the non cutaneous forms of melanoma ... for those melanomas that are growing in non cell-exposed areas like Anorectal melanoma ?

JNB: These tumours are very rare and it has taken a long time to generate information of value as a result. We know from our own studies that tumours arising in sun protected sites are more likely to be ulcerated (paper in preparation) and more likely to be thicker (possibly biological but also probably a function of late diagnosis). We reported in 2009 the observation that patients presenting with thicker primaries were more likely to have low serum vitamin D levels and that vitamin D deficiency was independently predictive of poorer survival [2]. Although it took some time these observations have been validated in other cohorts [3, 4], we continue to see this association in other data sets built in Leeds. We have more recently reported evidence for a causal relationship between reduced vitamin D/vitamin D receptor (VDR) signalling, increased β -catenin signalling and poor survival in melanoma [5]. In this paper we showed that melanomas arising in sun protected sites have lower VDR expression, and therefore these associations suggest that these tumors may be particularly aggressive as their protection from sunlight is associated with reduced VDR expression, increased β -catenin signalling and consequent greater proliferation and poorer immune responses as described in our paper [5].

We continue to work on samples from our Leeds Case control study to which participants were recruited with rare site tumours and will report on the genomic changes shortly. Others however have collaborated to generate larger data sets related to genomic characterisation of the commoner sub types especially acral lentiginous tumors. As expected the majority did not have the usual profiles of mutations seen in melanoma typified by C to T UV induced mutations, but a few did suggesting that a very small % of acral lentiginous melanomas are related to sun exposure [6, 7]. The mucosal and acral melanomas had different mutation profiles that did cutaneous tumours and had a higher frequency of chromosomal aberrations [7]. In other words, in many ways, these tumours are very different then to cutaneous tumours.

DW: From a molecular point of view, arguably the most comprehensive analysis was the article published a few years ago by Nick Hayward in Nature which mapped the genomic

architecture of the major melanoma subtypes, especially the non-cutaneous subtypes. I would refer you to that publication.

Hayward NK, Wilmott JS, Waddell N, Johansson PA, Field MA, Nones K, Patch AM, Kakavand H, Alexandrov LB, Burke H, Jakrot V. Whole-genome landscapes of major melanoma subtypes. *Nature*. 2017 May;545(7653):175-80.

**Q. Su Lwin: What is known about the mechanism of photoadaptation?
Epigenetic/immune regulation?**

JNB: as discussed in the Q&A session, as a non-photobiologist, I feel unable to answer this question. I have talked to photobiologists in the last few years about what is known about photoadaptation but as in the referenced review I believe that this is a complex area lacking in data [8] at least for human skin. There is a literature however on photoadaptation of species such as plankton. I would be delighted to hear from a photobiologist who would be able to enlighten me around current thoughts on photoadaptation in the context of skin cancer.

Q. Marta Szell: Does skin colour have any effect on the ratio of C to T transition in melanocytes and melanoma?

JNB: I am not aware of any data of this sort but this is outside my area of expertise

DW: that is an excellent question! Antony Young's group has done work comparing the DNA damage occurring in people with different skin types. The abstract is below. I highly recommend this article to you.

Fajuyigbe D, Lwin SM, Diffey BL, Baker R, Tobin DJ, Sarkany RP, Young AR. Melanin distribution in human epidermis affords localized protection against DNA photodamage and concurs with skin cancer incidence difference in extreme phototypes. *The FASEB Journal*. 2018 Jul;32(7):3700-6.

Epidermal DNA damage, especially to the basal layer, is an established cause of keratinocyte cancers (KCs). Large differences in KC incidence (20- to 60-fold) between white and black populations are largely attributable to epidermal melanin photoprotection in the latter. The cyclobutane pyrimidine dimer (CPD) is the most mutagenic DNA photolesion; however, most studies suggest that melanin photoprotection against CPD is modest and cannot explain the considerable skin color-based differences in KC incidence. Along with melanin quantity, solar-simulated radiation-induced CPD assessed immediately postexposure in the overall epidermis and within 3 epidermal zones was compared in black West Africans and fair Europeans. Melanin in black skin protected against CPD by 8.0-fold in the overall epidermis and by 59.0-, 16.5-, and 5.0-fold in the basal, middle, and upper epidermis, respectively. Protection was related to the distribution of melanin, which was most concentrated in the basal layer of black skin. These results may explain, at least in part, the considerable skin color differences in KC incidence. These data suggest that a DNA protection factor of at least 60 is necessary in sunscreens to reduce white skin KC incidence to a level that is comparable with that of black skin.

Q. Marta Szell to David Whiteman: When you did your model for decreasing the number of melanoma cases in the coming decades did you incorporate the factor of climate change?

DW: thanks Marta – no we did not factor in climate change. We used Age-period-cohort models to estimate the current trends in melanoma incidence, and then used those to

project forwards as the ‘best guess’ at what will happen in the future, under the assumption that ‘*things will continue to trend as they have been for the past 15 years*’. (This may be an unsafe assumption.) We then estimated the effect of increasing levels of sunscreen use, based on current levels of use in the Australian population and the effectiveness of sunscreen as reported in the Nambour RCT. This analysis therefore uses a measure called the Population Impact Fraction (PIF).

The article is referenced here:

Olsen CM, Wilson LF, Green AC, Biswas N, Loyalka J, Whiteman DC. How many melanomas might be prevented if more people applied sunscreen regularly?. *British Journal of Dermatology*. 2018 Jan;178(1):140-7.

Q. Enikö Sonkoly: Are there any genetic susceptibility factors for melanoma that affect the immune system rather than pigmentation (considering the success of immune therapies)?

JNB: As discussed in the Q&A session, not so far. We reported a pathway analysis of genes mediating immune responses which identified some pathways of significance in the training data set but these findings were not validated in the test data set [9]

1. Cust, A.E., et al., Associations of pigmentary and naevus phenotype with melanoma risk in two populations with comparable ancestry but contrasting levels of ambient sun exposure. *J Eur Acad Dermatol Venereol*, 2019.
2. Newton-Bishop, J.A., et al., Serum 25-Hydroxyvitamin D-3 Levels Are Associated With Breslow Thickness at Presentation and Survival From Melanoma. *Journal of Clinical Oncology*, 2009. 27(32): p. 5439-5444.
3. Wyatt, C., et al., Vitamin D deficiency at melanoma diagnosis is associated with higher Breslow thickness. *PLoS One*, 2015. 10(5): p. e0126394.
4. Fang, S., et al., Association of Vitamin D Levels With Outcome in Patients With Melanoma After Adjustment For C-Reactive Protein. *J Clin Oncol*, 2016. 34(15): p. 1741-7.
5. Muralidhar, S., et al., Vitamin D-VDR Signaling Inhibits Wnt/beta-Catenin-Mediated Melanoma Progression and Promotes Antitumor Immunity. *Cancer Res*, 2019. 79(23): p. 5986-5998.
6. Rawson, R.V., et al., Unexpected UVR and non-UVR mutation burden in some acral and cutaneous melanomas. *Lab Invest*, 2017. 97(2): p. 130-145.
7. Hayward, N.K., et al., Whole-genome landscapes of major melanoma subtypes. *Nature*, 2017. 545(7653): p. 175-180.
8. Garmyn, M., A.R. Young, and S.A. Miller, Mechanisms of and variables affecting UVR photoadaptation in human skin. *Photochem Photobiol Sci*, 2018. 17(12): p. 1932-1940.
9. Schoof, N., et al., Pathway-based analysis of a melanoma genome-wide association study: analysis of genes related to tumour-immunosuppression. *PLoS One*, 2011. 6(12): p. e29451.