# Student’s statistical significance probabilities analysis for several

Student’s T Test compares the significant differencebetween two groups (two means).

In this study, paired t-test is used to comparegroups and test the significant difference between two sets of data. If thedata are significant given by the P ?? 0. 05 were considered as significant data, P < 0. 01*, P < 0.

001**, P < 0. 0001***. The multiple t-test compares thestatistical significance probabilities analysis for several t-tests at once. Thetwo-way ANOVA used to compare independent variables of interest and to understandif there is an interaction between them in different conditions. Our hypothesis findings needed more common hypothesistests such as two-way analysis of variance ANOVA. In this study, we mainly havetwo independent factors that are autophagy and IR with different time points.

Thisbasic research begins with a question that whether autophagy inhibition is moreeffective for the PCa patients’ treatment combined with radiotherapy (RT) ratherthan RT treatment alone. To test this question, we need to transform basicquestion to a testable hypothesis, labeled H0 named as a Nullhypothesis, which takes the following form: H0: Whether autophagyinhibition is NOT more effective for the PCa patients’ treatment combined with RTrather than RT treatment alone. To test this hypothesis, we harvested thesamples from PCa cell lines as explained in (2. 2 Cell culture and treatments)and measured the results in order to decide whether the data from thatexperiment provides a strong evidence in order to reject the H0 ornot. If our evidence is strong to reject H0, then we are indirectlyaccepting the alternative hypothesis (Ha), which is: autophagy

inhibition ismore effective for the PCa patients’ treatment combined with RT rather than RTtreatment alone.

For each experiment, we collected the samples data to defineour hypothesis involving its finding by using the decision rule whether rejectthe null hypothesis or not. The null hypothesis is rejected if the p-value isless than the significance level, ?. ? is called the significance level, and is the probabilityof rejecting the null hypothesis given that it is true (a type I error). It isusually set at or below 5%. and the p-value is a number between 0 and 1 andinterpreted in the following way: A small p-value (typically ? 0.

05) indicatesstrong evidence against the null hypothesis, so we reject the null hypothesis. In student’s (paired) t-test, computed data of the difference between twosamples before and after IR treatment were as followed: calculating the mean bycounting foci numbers/ nuclei, that included > 30 foci/field. Each experimentwas repeated 3 times as indicated by (n= 3), to allow calculation of the averagemean of the gathered data. For example, H0: autophagy has no role on theDNA-damage response (DDR) signaling in response to ionizing radiation (IR)treatment.

In contrast, Ha: autophagy regulates the DDR signaling in responseto IR treatment; we examined it in autophagy-deficient PCa cells. Immunostaining showed that the number of ? H2AX IR-induced foci (IRIFs) at 0. 5hwere not significantly different between dox-pretreated cells followed by IRcompared to IR treatment alone in LNCaP (Fig 3. 2.

a and b). To explain itstatistically, the probability of forming ? H2AX foci is 0.

0955, which is largerthan 0. 05, that leads to decreased evidence against

H0. However, autophagy-deficient cells revealed persistent ? H2AX foci at 24h following IRtreatment compared to the parental cells following IR alone.

The probability ofwhich is <0. 0001, this is much lessthan 0. 05, hence the evidence against H0 is strong and it can berejected. Under the assumption that the null hypothesisis true, we repeated large number of random samples (> 30 foci/field) to testH0 and Ha.

The significance level (a)= 0. 05, which indicates 5% of the difference exists in the distribution. We canalso see if it is statistically significant using the other common significancelevel of 0. 01. As a result, our average mean didn’t fall within thesignificance region, which led us to accept the null hypothesis. However, theprobability and the significance level represent the likelihood of finding asample mean that would set in both tails of the distribution.

Hereafter, thesignificance levels and P values are key tools, that helped us to measure and decreasethis type of error in our hypothesis test. All assumptions should include appropriate positive andnegative controls. It is also valuable to distinguish between assessments thathave a reproducible quantitative readout on how data will be tested acrosstreatment groups for significance, and rules for data exclusion. Indeed, it isdifficult to predict a scenario where this would not benefit scientific rigor, replicabilityand reduce bias. One possible that needs to confirm biological replicates byusing different samples are independent from another lab.